Evaluation of a Laser Fluorescence Device for the Detection of Demineralisation

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Abstract

Early, accurate caries detection is essential for the provision of effective dental care and the prevention of the potentially painful sequelae of undiagnosed or untreated disease. Despite a reduction in caries prevalence, detection of caries remains difficult and existing techniques are associated with a number of disadvantages. A new device known as 'DIAGNOdent' has been developed with the aim of overcoming some of these difficulties and the manufacturers claim its ability to detect and quantify demineralisation by virtue of altered fluorescence in carious tooth tissue. This thesis describes a series of experiments which aimed to evaluate the DIAGNOdent device and investigate some aspects of its detection of demineralisation.

The ability of the DIAGNOdent to detect and quantify enamel demineralisation in various laboratory created carious lesions was assessed and results indicated that the device was unable to accurately effect this. Comparison was made with another fluorescence based caries detection device which demonstrated a superior performance in this respect. The influence of exogenous staining on the DIAGNOdent analysis of the same laboratory created lesions was investigated and found to have a profound effect on the instrument's ability to detect and quantify the degree of demineralisation. Subsequent work, however, determined that the effect of the exogenous staining was unstable beyond an initial period of observation.

Further studies examined the ability of the DIAGNOdent to detect residual caries at the margins of amalgam restorations. Results using this model indicated that the use of the DIAGNOdent offered no significant advantage over visual examination alone.

In a further experiment, the distribution, within natural carious lesions, of the fluorescence measured by the DIAGNOdent was investigated and the DIAGNOdent assessments of the lesion related to a visual assessment of caries extent. Both transverse and longitudinal mapping of fluorescence was carried out but results failed to demonstrate a clear pattern of fluorescence distribution within the lesion. In addition, no significant correlation was found between the visual and DIAGNOdent assessment of the lesion.

The results of these experiments question the ability of the DIAGNOdent to detect caries by recognising demineralisation and would seem to suggest its mode of action to be related to detection of other fluorophores within the lesion. The lack of relationship between the DIAGNOdent and clinical assessment of a lesion along with its apparent limitations in respect of caries detection at restoration margins urge caution in using the device clinically. Further *in vivo* work is required to determine the applicability of these findings to the clinical situation.

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Declaration

I, Gavin Alexander Welsh, declare that the following thesis is entirely my own work and composition.

Signed:

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

1.1.1 Definition of Dental Caries

Dental caries has been defined as 'an infectious disease causing localised destruction of the dental hard tissues by acids in the microbial deposits adhering to the teeth.' (Fejerskov and Thylstrup 1986)

The 'localised destruction' of the teeth described in this definition is the principle sign of the disease and early recognition of this is critical in diagnosis. The relative complexity of the interactions between the micro-organisms and the oral environment along with the physical characteristics of the tooth surface however, result in some practical difficulties in achieving this aim. The simple visual recognition therefore, of this clinical sign is not sufficient and adjuncts to aid earlier detection are desirable.

1.1.2 Prevalence of Dental Caries

Dental caries exists throughout the world although the prevalence and severity of the disease varies between different populations (Marthaler et al., 1996). The proportion of people affected may differ as well as the number of teeth showing signs of carious attack in any one individual.

The National Surveys of Adult Dental Health have been undertaken since 1968 and have provided a 10-yearly summary of the clinical dental condition of adults in the United Kingdom. The fourth of these national surveys was published in 2000, based on data collected in 1998 and this provides the most reliable and up-to-date picture of caries prevalence in the United Kingdom. (Kelly 2000)

In common with most westernised countries, oral health has generally improved in recent years (Kelly 2000) and the data from the most recent Adult Dental Health Survey in 1998 indicate a decreased prevalence of dental caries in comparison to previous surveys. Overall, 24% of dentate adults had visually detectable primary (untreated) caries. 22% had primary cavitated lesions and 8% had evidence of recurrent caries. The average number of decayed teeth in any individual was 1.1 compared to 1.6 in the 1978 survey.

The experience of caries was shown to vary between different age groups with younger individuals showing the highest prevalence; 25-34 year olds demonstrated the highest disease prevalence of all with 35% showing signs of primary visual decay and 26% with cavitated lesions. The survey also reported on the distribution of caries; molar teeth were most affected by disease and 85% of these were either missing, decayed, unsound or restored and 10% of molars had evidence of untreated caries.

The distribution of caries experience within the population is not uniform and poorer socio-economic groups and certain geographical areas showed markedly increased prevalence. It is clear that dental caries is a significant problem affecting a large proportion of the population of the United Kingdom.

1.1.3 Diagnosis of Dental Caries

Traditional techniques for the diagnosis of dental caries have involved visual inspection of the tooth surface and radiographic examination. Although they form the mainstay of caries diagnosis in routine clinical practice, these methods are associated with a number of problems (Stookey et al., 1999).

Early detection of the carious lesion is desirable at a point when changes in diet and/or oral hygiene habits in combination with optimal fluoridation may stop the progression of a lesion or even allow its remineralisation (Dijkman et al., 1986). However, significant demineralisation of enamel and dentine beyond this point may have already taken place before a lesion becomes clinically detectable by traditional diagnostic methods as they lack sufficient sensitivity to detect the early changes of the carious process (Wenzel et al., 1991, Lussi 1993). Furthermore, none of the traditional diagnostic methods has the ability to account for the dynamic nature of the carious process including the possibility of reversal: clinically, dentists are forced to measure a continuously dynamic process as simply a subjective presence or absence of disease.

This inability to reliably quantify dental caries must be considered a major disadvantage in planning appropriate treatment and by the time of detection, arresting or remineralisation of a lesion is often not possible and the downward spiral of restorative therapy is commenced. Notable improvements in restorative dental materials have taken place over recent years but even the most durable materials have a limited longevity in service and will often require multiple replacements during the patient's life (Elderton 1994). Therefore, early detection of carious lesions has the potential to allow a more preventative approach to the management of caries rather than the current invasive repair of the effects of the disease.

The greater conservation of tooth tissue afforded by early and accurate diagnosis as well as the ability to employ conservative, monitoring techniques rather than invasive restoration has a number of advantages. Dental caries and its sequelae are associated with significant patient morbidity and unfortunately for many, remedial treatment presents a major psycho-social problem. The advantage of avoiding the pain of acute pulpitis is obvious but a reduction in the need for operative dental treatment is also an advantage from the patient's perspective. In addition, restorative treatment of dental caries *de novo* and subsequent replacement and maintenance of restorations place a notable financial burden on provision of healthcare.

Techniques to allow accurate and early diagnosis of dental caries are certainly desirable and a number of 'alternative' techniques have been developed in recent times in an attempt to achieve this goal. Some of these alternative techniques of

caries diagnosis have been available for many years but few have proved to be of either sufficient practical suitability or clinical validity to be readily accepted into everyday practice (Verdonschot et al., 1992). No single method offers a perfect, simple and reliable diagnostic test and each must be used in conjunction with sound clinical judgement. Nevertheless, one or more of these methods, with further refinement, may provide dentists with a solution to an increasingly difficult problem.

1.2 Models for the Study of Caries

A model is a process which simulates a real phenomenon. It may be useful in research since study and analysis of the model may allow an increased knowledge and understanding of the real phenomenon. Dental caries has been studied via a variety of models.

1.2.1 *In Vitro* Studies

In vitro studies of dental caries have involved the use of whole extracted natural teeth which demonstrate naturally occuring carious lesions (Brinkman et al., 1988). Alternatively the extracted teeth may be used to provide suitable enamel/dentine specimens for the creation of artificial carious lesions. *In vitro* methods are generally the least expensive and least time consuming of the methods of modelling the caries process. The conditions of *in vitro* study are more readily controlled in that they allow quite specific investigation of a particular variable e.g. pH, fluoride concentration while others are held constant (Mellberg and Mallon 1984).

In vitro test methods have been developed beyond the point of simple analytical studies on the static carious lesion and using the 'pH cycling' models, described by Ten Cate and Duijsters (1982) it is possible to study dynamic mineral loss and gain in demineralisation/ remineralisation studies.

By definition, *in vitro* models use enamel/dentine specimens which are separated from the oral environment and accordingly have limitations in terms of their ability to simulate the caries process. Ten Cate (1990) reported that important microbiological factors and specific influences of saliva cannot be adequately simulated *in vitro*. Luoma et al., (1983) reported however, that there have been some attempts at developing models which aim to incorporate the effects of such factors.

1.2.2 *In Situ* Studies

In situ models of dental caries have used mineralised tissue samples of various origins e.g. extracted human teeth or bovine enamel slabs mounted in some form of appliance to retain them within the oral cavity during the period of study. These studies have been designed to further understanding of the caries process within the complex environment of the oral cavity. Current *in situ* techniques have utilised the bonding of orthodontic brackets incorporating a test specimen and plaque retentive gauze onto the buccal surfaces of molar teeth. Alternatively, test specimens have been incorporated into custom made removable denture type appliances (Wefel 1990).

In situ models may provide the advantages of controlled conditions of *in vitro* systems using well defined substrates whilst examining the carious process taking place in the natural environment of the human mouth. Direct effects of saliva and plaque are therefore accounted for along with other potential influences such as

toothbrushing and mastication. Nevertheless, *in situ models* are still simulations of the caries process studying demineralisation and remineralisation rather than natural caries *per se*. The duration of the *in situ* trials and the nature of the system often result in 'subclinical' lesions which although useful for analysis by laboratory techniques, are of questionable clinical significance (White 1992). Other factors which deviate from the normal clinical situation of *in situ* studies include the artificial plaque retention using orthodontic bands or gauze which can alter the microflora significantly in comparison to the natural tooth surface (Boyar et al., 1989).

1.2.3 *In Vivo* Studies

In vivo studies of dental caries have involved investigation of naturally occurring lesions on vital teeth with an intact pulpo-dental complex and a natural covering of pellicle and plaque. This is caries as it occurs naturally in the oral cavity and is therefore not a model of the carious process but is the real event itself.

Ethics issues are central when attempting to study caries *in vivo* and it is not appropriate to deliberately initiate caries or allow existing carious lesions to progress beyond their earliest pre-cavitation stages. Therefore, data from *in vivo* studies of dental caries are limited. However, some studies of caries *in vivo* have been carried out in the past and although ethically inappropriate to repeat now, studies such as the Vipeholm Dental Caries Study (Gustafsson et al., 1954) have provided valuable

advances in the understanding of dental caries. Today epidemiological studies such as the Adult Dental Health Surveys (Kelly 2000) study caries *in vivo* but are limited to prevalence studies and are not interventional in nature and therefore do not provide information on the caries process itself.

Historically, animal models have been used to study caries *in vivo* (Navia 1977). These have continued to evolve and are currently widely used in caries research, their advantages being reported as speed, economy and ease of performance along with the excellent simulation of the caries process (White 1992). Caries induced in rats demonstrates many histological features in common with human caries and rat saliva has also been reported as being responsible for a protective pellicle on the tooth surface which influences the demineralisation/remineralisation process (Briner 1981). Nevertheless, animal studies are not perfect models for human caries and differences in behavioural characteristics may account for some inconsistencies between results from animal studies and subsequent clinical trials (White 1992).

1.3 The Artificially Created Carious Lesion as a Model for studying Dental Caries

The preparation of artificial carious lesions in enamel must be considered important in many areas of caries research. Silverstone (1977) reported that despite its apparent suitability, the naturally occurring incipient white spot lesion is too variable in structure and often too far advanced to be used for precise studies of demineralisation and remineralisation. In many cases, the white spot lesion with its sound outer fluoride rich layer represents an advanced stage of caries formation already affected by external influences such as topical fluorides (Ingram 1983). Therefore, artificial lesions, simulating various stages of the caries process are often used as an alternative in many quantitative *in vitro* studies of the caries process.

1.3.1 Creation of Artificial Carious Lesions

Early studies designed to produce artificial carious lesions have employed bacterial plaque grown *in vitro* on extracted teeth (Enright et al., 1932). Models for producing caries-like lesions using oral bacteria have endured until more recently (Clarkson et al., 1984) but long before, it was realised that bacteria were not necessary for the production of demineralised lesions and techniques to produce lesions using artificial demineralising solutions were developed.

A variety of different demineralisation systems has been used for the preparation of such artificial lesions in enamel. Theuns et al., (1983) described three main types:

1. Gel systems
2. Surface protection systems
3. Buffer systems

Successful production of artificial lesions has been reported using all 3 methods but differences in the resultant lesions have been highlighted (Manson-Hing et al., 1972). The production and subsequent behaviour of the lesion can be influenced by careful control of the pH and the ionic constituents of the demineralising system (Groenveld and Arends 1975).

1.3.2 Gel Systems

The key element in such systems is the presence of a surface protective agent to simulate the action of salivary pellicle in preventing the etching of the enamel surface during demineralisation (Zahradnik et al 1976). In gel-based systems, organic polymers such as gelatine, hydroxyethylcellulose and methylcellulose act in this way (White 1987). However, White (1987) has described the disadvantages of these systems including the variability of their composition and the presence of relatively high levels of impurity in the commercially available gel preparations. The presence of these unknown chemical agents is a disadvantage of these systems as they may

affect the results when resultant lesions are used experimentally. In addition, Pearce (1983) has suggested that experimental work using such lesions may be less reproducible.

1.3.3 Surface Protection Systems

Alternative systems with known, added surface protective agents have been described by Featherstone et al., (1979) who described the use of methanehydroxy-diphosphonate (MHDP) as a surface protectant along with other macromolecules; polyphosphates and phytate to prevent surface etching during demineralisation. However, low molecular weight MHDP may diffuse into the carious lesion during its creation and this may potentially influence subsequent lesion behaviour in remineralisation studies (Francis 1969). White (1987) reported his use of a high molecular weight polycacrylic acid (Carbopol C907) as a surface protectant after recognising problems associated with existing systems. This is discussed further in section 1.3.5.

1.3.4 Buffer Systems

In buffer systems, solutions of accurately known composition and, in particular, a well defined degree of undersaturation, with respect to hydroxyapatite, are used to bring about demineralisation. Calcium and phosphate ions are often added to the demineralising solution to alter the relative undersaturation of the demineralising

medium with respect to hydroxyapatite. This has been shown to influence ionic movements and promote the subsurface dissolution of enamel which is characteristic of natural lesion progression rather than simple surface dissolution (Theuns et al., 1983). Theuns et al., (1983) studied the effect of altering the demineralising solution concentration of these ions on the mineral content of the surface and body of lesions and also the lesion depth. Microradiographic analysis of lesions created on extracted premolars suggested that, independent of pH of solution or time of exposure, an increase in the degree of undersaturation of the demineralising solution resulted in a decrease in the mineral content of the surface and body of the lesions produced. In addition, the speed of demineralisation appeared to increase with increased undersaturation of the solution.

Fluoride ions have also been added to the demineralising solution to help prevent surface etching of the enamel and promote subsurface demineralisation (Larsen 1974). Pearce (1983) however, described the formation of a surface deposit on the lesions produced by media including fluoride and suggested problems with subsequent analysis caused by this. The author further stated the potential for problems associated with the very high fluoride concentration in the final lesions which would have the potential to influence the results of de/remineralisation studies.

1.3.5 The 'Carbopol' Demineralising Solution

White (1987) described the use of a demineralising medium based on Carbopol C907, a synthetic high molecular weight polyacrylic acid as a surface protective agent and lactic acid as a demineralising agent. This demineralising solution was used to create smooth surface lesions on the buccal surfaces of extracted teeth and these lesions were analysed by surface micro-hardness, polarized light microscopy and microradiography.

The results indicated that this medium was effective in protecting the enamel surface resulting in caries like lesions. It was suggested that the adsorption of polyacrylate onto the apatite crystals on the enamel surface is responsible for preventing surface etching by acting as a dissolution inhibitor and that the higher molecular weight of the Carbopol in comparison to other surface protectants eg. MHDP, may prevent its subsequent diffusion into the carious lesion. As with other described demineralisation systems, the addition of calcium, phosphate and fluoride ions to the solution had the effect of increasing the surface hardness of the lesion and enhancing the subsurface demineralisation. In this way the rate and degree of demineralisation were within operator control and furthermore, the histology of the resultant lesions was 'lifelike' in comparison to naturally occuring carious lesions. In addition to the apparently favourable lesions produced, the synthetically prepared Carbopol was relatively free from impurities which may potentially have influenced demineralisation/ remineralisation in other systems.

1.3.6 The 'Glasgow' Demineralising Solution

A buffered solution system based on acetic acid was developed for production of artificial caries lesions in the laboratories of Glasgow Dental School (Damato et al., 1988). These workers compared their solution with the acidified gelatin system described by Silverstone (1966). In subsequent demineralisation studies, lesions prepared by the acid-buffered solution demineralised to a greater extent than crude gelatin lesions. Damato et al., (1988) suggested that the high mineral ion impurity of crude gelatin may interfere with the processes of ionic exchange. In addition, the authors showed that undialyzed gelatin contained 0.15ppm fluoride which may result in very high fluoride concentrations in the lesions making them resistant to demineralisation. This is in agreement with the findings of Pearce (1983) who also studied the fluoride profile in enamel after exposure to various artificial caries producing systems. Although Damato et al., (1988) reported that this method of lesion preparation had an effect on the subsequent behaviour of the lesion, subsequent studies by Damato et al., (1990) have shown that the acetic acid based buffered solution described has been used successfully to create lesions for caries research.

1.4 Techniques for Evaluation of Demineralisation

A number of experimental techniques exists for the evaluation of demineralisation (and remineralisation) of tooth specimens in cariology research (Arends and ten Bosch 1992). It may be considered desirable, for any such technique, to be able to quantify the loss or gain in mineral as a result of a process or experimental technique and also, in some situations, to be able to identify specifically where in the specimen the mineral loss or gain has taken place.

1.4.1 Microradiography

Mineral quantification by means of x-ray absorption has been known for at least 60 years being first described by Thewlis (1940). Since then, techniques have been refined and three different microradiographical techniques are now available:

1.4.2 Transverse Microradiography

Transverse microradiography (TMR) or contact radiography is the most commonly employed experimental technique for evaluation of demineralisation. In this technique, the sample for investigation is first prepared into a thin section of 120-140 μm. Sections are prepared planoparallel and orientated at right angles to the anatomical tooth surface. The slices are placed onto a piece of x-ray film along with an aluminium calibration wedge and exposed to monochromatic x-rays. The degree

of mineralisation of the section affects the absorption of the x-rays; this is directly reflected in the optical density of the developed x-ray film.

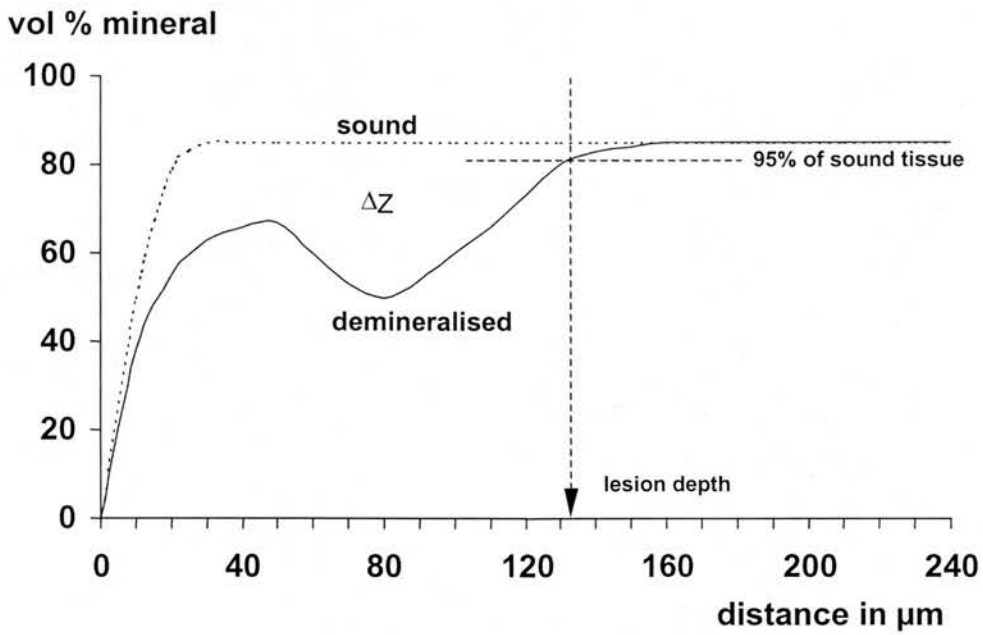
Developed microradiographs are subsequently analysed by microdensitometry. The microradiographic image is illuminated with a precisely controlled, constant and uniform light source and the light transmitted by the microradiograph passes to a detector and recorder. Thereafter, the amount of transmitted light detected is a reflection of the degree of mineralisation of the original specimen at that point. Calibration is effected via the aluminium step-wedge which provides a reference image of known radiopacity. Calculations to describe and quantify the mineral loss are then possible.

Ten Bosch and Angmar-Månsson (1991) described 3 units to express mineral loss from enamel.

1. Mineral loss from a specific point expressed as a decrease in the mineral concentration relative to sound tissue. This mineral loss value has the unit % volume or $kg.m^{-3}$.
2. The integral of change in mineral content over a known distance from the tooth surface. This is expressed in $kg.m^{-2}$ or vol%.µm.
3. Total mineral loss expressed in g. or kg.

Angmar et al., (1963) described the use of a formula to calculate the decrease in mineral concentration of the specimen which is expressed as % volume or $kg.m^{-3}$. A plot of volume percentage of mineral against distance from the outer surface of the lesion can then be made (figure 1.1), and from this, the lesion depth (L_d) is calculated by measuring the distance from the outer surface to the point where the mineral content is 95% of the sound tissue. In addition the mineral loss for the lesion (ΔZ) may be determined by measuring the area between the obtained plot and the extrapolated plot for sound tissue. An additional parameter sometimes quoted in TMR studies is the R value, $R=\Delta Z/ L_d$ (in vol%) (Arends and ten Bosch 1992).

Figure 1.1. Stylised plot of volume percentage of mineral against distance from the outer surface of a demineralised lesion.



Today, computerised microdensitometry is routinely available and the transmitted light form the microradiograph may be recorded by a video camera and frame-grabber attached to a microcomputer. Dedicated microdensitometry software (Transverse Micro Radiography (TMR) v1.25e, Inspecktor Research Systems BV, Amsterdam, The Netherlands) may then be used to carry out all calculations and plotting.

The principle advantages of TMR as a method for quantifying mineral loss in a specimen have been described as its accuracy and ability to measure the distribution of mineral loss (Arends and ten Bosch 1992). They reported the accuracy of TMR for dentine to be +/- 5μm for lesion depth and +/- 140 vol%.μm for ΔZ. They described the major disadvantage of the technique as the destruction of the specimen in preparation for TMR. Furthermore, Arends and ten Bosch (1992) reported the distorting effect due to x-ray absorption of ions such as tin from stannous fluoride present on the lesion surface. This, they suggested, can lead to misinterpretation as remineralisation.

1.4.3 Longitudinal Microradiography

Another microradiographic technique, longitudinal microradiography, has been described by de Josselin de Jong et al., (1987) whereby longitudinal sections, 0.5mm thick, are cut ***parallel*** to the tooth surface. As with TMR, the sections are exposed to x-rays on photographic film along with a calibration wedge and then developed in the

normal way. The developed film is scanned by computer measuring the optical transmission at each point on the scan. The authors suggest a major advantage of this technique is the preservation of the sample following analysis allowing several sequential measurements if desired. However, they add that exposure to X-rays of the anatomical surface of the lesion rather than a cross-section (as in TMR) means it is not possible to derive information on variation of mineral content at various depths through the lesion.

1.4.4 Wavelength Independent Microradiography

Herkströter et al., (1990) described this technique for the non-destructive measurement of the mineral content of whole teeth. This technique used polychromatic high-energy X-rays (up to 60kV) and the authors suggest that this is able to produce non-destructive determination of the mineral content of whole teeth. However, they suggested that this technique was most suitable for enamel and dentine samples between 0.3 and 6mm thick and with these specimens produced reasonable detection limits. However, when whole teeth are used, the detection threshold of 0.05kg.m^{-2} or 1500 vol%.µm is much lower than for TMR or LMR.

1.4.5 Other Techniques

A range of other methods used for quantification of mineral content has been described. However, none of these other techniques is in common usage in cariology

and microradiography remains the principle method of evaluation of the mineral content of a tooth specimen.

In iodine absorptiometry described by Almqvist et al., (1988), longitudinal tooth sections are exposed to photons from a source of ^{125}I. The initial and incident radiation energies are measured and the difference between the two is a measure of the calcium ion content of the tooth specimen under analysis. In this way, quantitative mineral loss and gain data of comparable sensitivity to LMR can be acheived.

Surface microhardness of specimens has also been demonstrated as a means of assessing the degree of mineralisation indirectly. (Arends et al., 1980). They investigated indentations of enamel surfaces produced under standardised conditions that could be measured microscopically with the magnitude of the indentation representing a measure of the mineral content of that specimen. Herkströter et al., (1989) considered that dentine specimens were not suitable for this technique due to the effects of distortion resulting from changes in the hydration of the specimen

Bakhos et al., (1977) reported the principle of iodine permeability of a lesion in assessing the mineralisation. Specimens were completely covered with Potassium Iodide solution for 3 minutes. Each specimen was then washed in water and the amount of iodine in the washing solution is measured by an iodide specific electrode. This has been shown to correlate with the degree of mineralisation of the specimen.

However, subsequent studies by Zero et al., (1990) have suggested only a weak correlation with the degree of demineralisation and have questioned the validity of the technique.

Arends and ten Bosch (1992) briefly discussed the use of wet chemical analysis involving the dissolving of specimens in acid before determining the calcium and phosphate content of the solution. However, they considered this technique rather insensitive with only a large loss or gain of mineral measurable.

1.5 Optical Methods for Detection of Dental Caries

Most presently used diagnostic methods for dental caries require visual observation of an optical signal. In the simplest form this may be unaided visual interpretation, with the clinician's eye, of reflected light from the tooth surface indicating changes in colour, texture and translucency of the tooth substance. However, enhancement of the technique has been made possible using both different light sources and more sensitive methods of interpretation of the resultant optical signal. This section of the literature review concentrates on such optical methods for the detection and quantification of caries.

1.5.1 Fiber-Optic Transillumination

The basis for this technique of caries detection is the lower index of light transmission of decayed tooth tissue in comparison to sound tooth structure. Thus when light is shone through a tooth, caries appears as a darker spot within the surrounding sound tooth structure (Stephen et al., 1987). The fiber-optic transillumination (FOTI) is performed with a fiber optic bundle light probe to deliver light to the tooth surface and is most commonly used in diagnosis of posterior approximal caries when the light probe is positioned on the gingiva below the cervical margin of the tooth, whereby the light passes through the tooth structures and approximal decay produces a dark shadow on the occlusal surface. The advantages of this technique are simplicity and its ability to be applied with clinical

equipment readily available in all dental surgeries. However, it is useful only for approximal surfaces and is unable to detect secondary caries (Stookey et al., 1999)

FOTI has been assessed as a method of detecting approximal caries. Peers et al., (1993) measured validity and reproducibility of unaided clinical diagnosis, FOTI, and bite-wing radiology in the diagnosis of approximal caries *in vitro*. Sixty models were made using extracted premolars and molars, each containing four teeth with six contacting approximal surfaces. The teeth were examined first using unaided clinical examination and then using FOTI. Bite-wing radiographs were then taken of the teeth set in the models. The three examinations were repeated after 1 week. Histological sections were prepared to give the valid state of disease in each surface. The diagnostic threshold was caries penetrating into dentine. The reproducibility of all three methods was acceptable with kappa values exceeding 0.6 and all specificity values exceeded 0.95. Statistically significant differences were seen between sensitivities for clinical and bite-wing diagnosis and between clinical and FOTI examination, but not between bite-wing and FOTI. This study concluded that the validity of FOTI is at least as high as that of bite-wing radiology, and both are superior to unaided clinical diagnosis.

In contrast, Choksi et al., (1994) carried out independent examinations of 300 patients to evaluate the performance of FOTI in caries detection. FOTI was used as an adjunct to clinical and radiographic examinations for caries, restoration or secondary caries of approximal surfaces in maxillary anterior permanent teeth. These

authors found clinical and radiographic examinations to be significantly more effective than FOTI.

Further evaluation of FOTI was carried out by Hintze et al., (1998) who investigated the diagnostic accuracy of visual, FOTI and bite-wing radiographic examination for the identification of cavitated carious lesions in contacting approximal surfaces. They also assessed the inter-observer agreement with these methods and with direct visual examination conducted after tooth separation. A total of 338 unrestored approximal surfaces in 53 patients were examined independently by 4 dentists using the 3 diagnostic methods. The results from the diagnostic methods were compared with the results from the direct visual examination for each observer. The sensitivities for identification of cavitated lesions using visual examination varied between 0.12 and 0.50. For FOTI and radiography, the sensitivities varied between 0.00 and 0.08 and 0.56 and 0.69, respectively. The specificities exceeded 0.90 for all observers with all methods. Kappa values expressing inter-observer reproducibility were lowest for FOTI, followed by visual and radiographic examination. On the basis of these results the authors concluded that FOTI was the least reliable of the diagnostic methods tested.

In a trial using FOTI for diagnosis in occlusal caries, Fennis-Ie et al., (1998) carried out a study to compare the performance of visual inspection, FOTI and electrical conductance measurements (ECMs) in predicting the onset of occlusal caries in children. 56 children, having first or second permanent molar teeth that were not

exposed to the oral environment for more than half a year, participated in the study. Following baseline data recording, the diagnostic measurements were repeated at 6-month intervals over a period of 2.5 years. During the period of the study, 220 of 652 sites were judged to have developed caries such that they required a sealant or a sealant restoration. Two examiners jointly decided on the decay status at the sites. Survival plots showed that ECMs were superior to FOTI and fissure discoloration in predicting the onset of occlusal caries and thus the researchers concluded that ECM is a better predictor of occlusal caries than fissure discoloration and FOTI, although the differences among the performance of the three methods in this study were very small.

Stephen et al., (1987) used FOTI during the course of a 3-year clinical trial which initially involved 3003 children. At the initial examination, a subgroup of 813 13-yr-olds, and at the following annual visit, 2247 14-yr-olds, were examined by FOTI using a 150 W lamp and 0.5 mm diameter probe. Routine clinical and radiographic examinations were performed separately. Compared to the clinical examination scores for anterior teeth, FOTI detected an additional 64% of interproximal lesions at the first visit while, for the larger number of children at the second visit, the increase in lesion detection level using FOTI was 37%. For the posterior teeth the comparable figure at the second examination was 92%. However, when FOTI data were compared to radiographic data for more than 52,000 posterior interproximal surfaces, FOTI could only detect between 17% and 48% of lesions depending on their extent.

On this basis, it was concluded that FOTI is not a suitable substitute for bite-wing radiography.

1.5.2 Digital Fiber-Optic Transillumination

This technique is a development from conventional FOTI. Light is delivered to the tooth surface from its source via fiber-optics and the scattering and changes in this light as it traverses the tooth are recorded as an image by CCD camera for subsequent analysis by computer software. The optical phenomena recorded are indicative of the carious status of the tooth under examination. (Stookey et al., 1999).

Initial studies have reported favourable results indicating the ability of this technique to detect incipient carious lesions *before* they become visible on radiographs (Schneiderman et al., 1997). In this study, 50 extracted teeth were examined by visual inspection, conventional radiographs and Digital FOTI. The computer software in the Digital FOTI system allowed the location and diagnosis of carious lesions by the operator in real time, and provided quantitative analysis for monitoring of the lesions. Using a gold standard of magnified visual inspection and histology when required, sensitivities and specificities were calculated for caries detection at approximal, occlusal, buccal/lingual and root sites. Sensitivity of Digital FOTI was far superior to conventional radiographs but specificity was slightly inferior. The greatest improvement in sensitivity was seen at buccal/lingual sites.

1.5.3 Light Scattering

ten Bosch et al., (1980) initially reported the principle of measuring optical scattering by a carious lesion as an indicator of its mineral content. They described the increased scattering and decreased absorption of light photons within a carious lesion due to the presence of relatively more water in comparison to sound enamel which they suggest results in the whiter, opaque appearance of the initial carious lesion in enamel. The observed whiteness was initially observed using a microscope and was correlated with mineral content.

Ten Bosch and his co-workers subsequently developed the Optical Caries Monitor (ten Bosch et al., 1984). The introduction of this device evolved the light scattering measurement technique into a more practical method involving an illuminating white light and a photodetector for quantification of emergent light incorporated into a handheld probe arrangement. This was tested in a trial involving artificially demineralised bovine enamel lesions and the collected light flux from the Optical Caries Monitor correlated with mineral loss as measured by both microradiography and atomic absorption spectrophotometry. A high correlation ($r=0.94$) was reported and the potential for development of this technique as a clinically practical tool was intimated by the authors.

Brinkman et al., (1988) evaluated the Optical Caries Monitor by assessing natural carious lesions in smooth surfaces of extracted teeth. They demonstrated that natural

lesions also reflected much more light than sound enamel. The depth of these lesions and their mineral loss were measured by microradiography. The correlation coefficient between mineral loss and the light scattering coefficient was 0.67 and the correlation between optical measurement and lesion depth 0.71. These results suggested a weaker ability to measure the extent of mineral loss than the results of ten Bosch et al., (1984) using artificially created bovine enamel lesions which the authors attributed to a greater natural variation within naturally occurring lesions and caused them to suggest some doubt over the clinical applicability of the technique.

Nevertheless, Ögaard and ten Bosch (1994) reported their use of the same method in an *in vivo* remineralisation study. White spot lesions induced by orthodontic bands were measured at intervals using the optical measuring device and the optical scattering co-efficient was demonstrated to decrease or return to baseline during a remineralisation programme. This, they regarded, was indicative of the quantitative ability of the technique. Despite these reports, the Optical Caries Monitor was not developed for everyday clinical use.

1.5.4 Light Induced Fluorescence

The English physicist Stokes, in 1852, was the first to systematically investigate the phenomenon of light of one wavelength being absorbed by a material and then re-emitted at a longer wavelength. The material used in Stokes' initial experiments was

the mineral fluorite and it is from this that the name given to the phenomenon, fluorescence, is derived.

Fluorescence of teeth has been recognised since early in the twentieth century with Benedict (1928) reporting the fluorescence of teeth under ultra-violet light. It was later, in 1933 that Eisenberg reported fluorescence of teeth as a result of stimulation by visible light with emission of a reddish coloured light as a result of far blue and violet excitation light.

In a short abstract, Naleway et al., (1979) reported the apparent decrease, under UV light, in the fluorescence of enamel in areas of reduced mineralisation such as carious lesions and developmental defects with the relatively demineralised area appearing as a dark spot against a fluorescent background. This seems to be the first recognition of any difference in fluorescence between sound and carious tooth substance.

Subsequently, Shrestha (1980) reported on the use of UV light in the detection of early stage smooth surface carious lesions. *In vitro,* the buccal/lingual surfaces of mandibular teeth of rats fed a cariogenic diet were examined under 366nm UV light using a stereomicroscope with a UV filter in the eye-piece. The number and location of carious lesions were noted. The same specimens were then re-examined under normal lighting conditions following staining with 0.06% murexide (acid ammonium purpurate). Significantly more lesions were detected using the UV method which led the authors to conclude as to the benefit of the UV light method for early caries

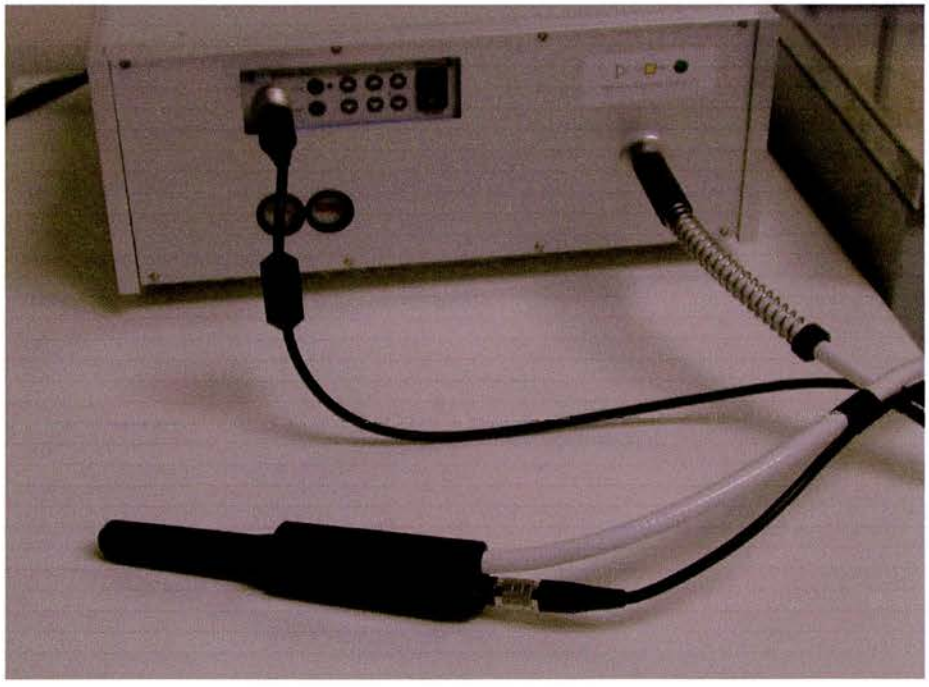
detection. Although the authors claimed that this method was working on the principle of UV excited fluorescence, it may be that this was not genuine fluorescence at all, but rather light scattering by organic material taken up by the early enamel lesion.

In 1981 however, the results of a systematic investigation of the fluorescence phenomenon in teeth were published. Alfano and Yao (1981) reported on 'Human teeth with and without dental caries studied by luminescent spectroscopy'. These workers used a tungsten light source to stimulate teeth with light of wavelengths 350nm, 410nm and 530nm. They noted that the peaks in the emission spectra were different namely 427nm, 480nm, 580nm. Also noted in this study was the shifting of the emission spectra of carious specimens towards the red end of the spectrum. The authors stated that this may therefore offer a non X-ray method of caries diagnosis and it is likely that this was the first published realisation of this important potential.

The technique of using the principle of fluorescence to provide a means of analysing and quantifying the carious status of a mineralised specimen has gradually evolved from this early work of Alfano and Yao (1981). The technique is now generally known as quantitative light-induced fluorescence or QLF. Although most subsequent development of QLF has been centred around stimulation with laser light, a commercial, clinically applicable device using a visible light source was developed.

The 'Clinical Caries Camera' (Inspektor Research Systems BV, Amsterdam, The Netherlands) was initially described using an argon laser light source (de Josselin de Jong et al., 1995) and in this form, is discussed further in the review of laser fluorescence of teeth (Section 1.5.5). The laser source was subsequently replaced by a filtered arc lamp providing a narrow band of visible light between 290 and 450nm and incorporated into a portable system for intraoral use (QLF \ clin Optical Measuring System, Inspektor Research Systems BV, Amsterdam, The Netherlands). The commercially available QLF \ clin device is illustrated in figure 1.2.

Figure 1.2 The QLF \ clin Device



Al-Khateeb et al., (1997a) evaluated this new device by comparison to microradiographic and chemical analyses of mineral changes in enamel during lesion formation and remineralization *in vitro.* The new device compared favourably with the argon laser version of the device. A significant correlation was found between fluorescence changes and mineral loss: r = 0.79 (laser system) and r = 0.84 (portable lamp system). The correlation between the arc lamp and argon laser devices was r = 0.93. On this basis, the new portable fluorescence device was deemed to be a promising new tool for reproducible and sensitive assessment of the severity of incipient enamel lesions.

1.5.5 Laser Induced Fluorescence

In addition to conventional, lamp light sources, laser light has also been studied in respect of the fluorescence phenomenon. Visible laser light within the blue-green region has been used as the light source for the development of techniques for detection of carious lesions since the early 1980s.

Bjelkhagen et al., (1982) used an argon-ion laser to illuminate teeth with blue-green light of 488nm. With their device, the laser beam could be spread by a lens to illuminate an area corresponding to 2-3 clinical crowns at a time or it could be delivered to the tooth surface via fiber-optics in a probe type device. The fluorescence of the specimen under study was observed and photographed with the aid of barrier filters. Intact enamel fluoresced with a yellowish light when stimulated

by the 488nm light source and both incipient and developed enamel carious lesions were visible as dark areas which contrasted against the surrounding sound enamel. The darker appearance of the lesions was attributed to a decreased fluorescence of the carious relative to the sound tissue. In contrast, the difference in the fluorescence of sound and carious dentine was far less marked and proved to be barely perceptible by the technique of Bjelkhagen and co-workers. Micro-radiographic analysis of longitudinal sections of the fluorescent lesions was also carried out and this appeared to confirm the correspondence of the dark area in the laser fluorescence with the demineralised zone in the enamel lesion. Using the same fluorescence technique, these researchers were also able to demonstrate occlusal and approximal lesions at a stage when they were not evident clinically or radiographically.

Later, Sundström et al., (1985) published results of their laser induced fluorescence of sound and carious tooth substance. In this study, fluorescence spectra were recorded of dentine and enamel illuminated with laser light of wavelengths of 337, 488, 515 and 633nm. The fluorescence obtained by illumination with UV laser light at 337nm had a peak at about 400nm in both enamel and dentine. Compared to intact enamel, the fluorescence from enamel with initial carious lesions was of lower intensity and had a slight red shift. Illumination at 488nm produced fluorescence with a peak at about 540nm in dentine as well as enamel. The difference in the intensity of fluorescence between sound and carious enamel was generally greater at this wavelength than at any of the others tried, and the red shift from the carious enamel was also more pronounced. Illumination at 515nm produced fluorescence of

similar wavelengths but with much less difference between intact and carious enamel. No fluorescence within the visible range was obtained by illumination with a low power He-Ne laser at 633nm. As a result of examining these different excitation wavelengths, the authors concluded that illumination at 488nm was most suitable for the detection of initial carious lesions by the fluorescence technique.

The laser fluorescence method of monitoring caries was progressed and developed for *in vivo* assessment by de Josselin de Jong et al., (1995). They used an argon-ion laser to illuminate the tooth surface via an optical fiber probe delivery system. A colour charged coupled device (CCD) camera was incorporated into the probe tip and a high pass filter placed in front of this to cut out light with wavelength less than 520nm. In this way, the video camera was able to record the fluorescence image of the tooth surface. A computer with dedicated software was used to display the real time image on screen. Incipient enamel lesions were seen as areas darker than the surrounding enamel.

Fluorescent images obtained in this way were stored on the hard disc of the computer for subsequent analysis. The decrease in fluorescence radiance relative to the surrounding sound enamel at individual pixel points was quantified and the lesion size calculated by measuring the surface occupied by points with a fluorescence radiance difference greater than 10% relative to the surrounding sound enamel. Other parameters recorded were mean and maximum changes in fluorescence for the lesion. It is suggested that these data may be used to estimate the degree of mineral loss and

furthermore lesion depth as a direct relationship between these parameters was suggested by Hafström-Björkman in her *in vitro* assessment of laser fluorescence Hafström-Björkman et al., (1992).

In practical terms, the design and set up of the laser fluorescence device has an effect on the quality of the resultant image and therefore on the diagnostic ability of the system. In particular, the means of delivery of the light into the tooth is important. In an *in vitro* study, Lagerweij et al., (1999) compared three different light-induced fluorescence systems;

- A water cooled argon laser with ring illuminator
- An air cooled laser with beam splitter
- An arc lamp with liquid light guide

These are described in this paper and the three systems compared in their ability to detect small enamel lesions. The repeatability of the three systems was also tested. Forty human enamel specimens were mounted in acrylic and polished. Each specimen was individually exposed to Carbopol demineralizing solution for 24, 48, 72 or 96 hours. The mineral loss of the 40 specimens was assessed with blue-violet light-induced fluorescence. Each image was captured with a video camera and analysed with dedicated software with the measurements repeated 3 times with complete shut-down of the system in between. The same measurements were performed with the ring illuminator, the beam splitter and the arc lamp. The

specimens were then cut into thin sections and analysed with microradiography to serve as the gold standard. Similar high correlations between microradiography and the light-based analysis systems were found for the beam splitter and the arc lamp set-up but the ring illuminator displayed a poorer correlation. The repeatability was similar for the three techniques but was best for the beam splitter set-up. The authors suggested that this indicated that the light-induced fluorescence measurement technique can be used in different configurations but that the repeatability and validity of the measurements may be influenced by the physical stability of the set-up.

1.5.5.1 Evaluation of Laser Fluorescence Devices

As early as 1985, Hafström-Björkman and co-workers carried out a clinical trial comparing the conventional clinical examination using mirror, probe and bite-wing radiographs with a laser fluorescence examination (Hafström-Björkman et al., 1985). This trial was reported only in abstract form but described the use of a 488nm argon-ion laser to examine the teeth of high caries activity school-children. It was reported that more caries-like changes were detected in enamel with the laser fluorescence method than by the standard clinical examination. However, no further details were provided.

Hafström-Björkman et al., (1992) assessed the ability of laser fluorescence measurements to quantify mineral loss in an *in vitro* caries model. Artificially created

enamel lesions were monitored over a 9 day period and were assessed by both laser fluorescence and microradiography. Results of this trial indicated a very strong correlation between the two methods of quantifying mineral loss (r=0.97). In addition, the discriminatory threshold for the laser fluorescence technique was lower than for microradiography indicating its ability to detect caries at an earlier stage. The authors therefore concluded that this was a useful, repeatable and sensitive method for quantifying mineral loss and suggested the potential development of the technique for diagnosis of caries *in vivo*.

An *in vivo* laser fluorescence system was described by de Josselin de Jong et al., (1995). The device was evaluated in a clinical trial involving creation of enamel lesions on buccal surfaces of teeth scheduled for extraction. The lesions were assessed by laser fluorescence over a 5 week period and significant differences in recorded parameters were noted at different time points. This was suggested to offer evidence of the suitability of the technique for monitoring mineralisation changes *in vivo*.

Emami et al., (1996) studied naturally occurring incipient enamel lesions for mineral loss using a laser fluorescence method and compared the results to assessment of mineral loss made by microradiography. Extracted teeth were selected for early enamel lesions on the buccal surface and enamel slab specimens were prepared for analysis. Specimens were analysed by 488nm argon laser and computer software used to calculate the mean loss in radiance for the lesion relative to sound enamel. In

addition, longitudinal microradiography analysis was carried out for each specimen. The correlation between the two methods was reported at r=0.73 which is lower than that reported in the *in vitro* caries model using artificially created lesions Hafström-Björkman et al., (1992). This may indicate a potentially problematic difference between the naturally occurring and artificially created lesions for analysis by laser fluorescence.

Subsequently, Hall et al., (1997) also aimed to demonstrate the quantitative nature of laser fluorescence (LF) by means of comparison with transverse microradiography (TMR). This study was carried out using artificially created lesions in bovine enamel. The results demonstrated reasonable correlation between both the histological depth and mineral loss measured by TMR and the percentage change in mean fluorescence radiance measured by LF (r = 0.70 and 0.83, respectively). In addition, these workers also attempted to determine the ability of LF to detect caries adjacent to amalgam restorations. The detection and quantification of mineral loss adjacent to amalgam restorations may have the potential for the management of recurrent caries. Using artificially created lesions on extracted human molar teeth, they were able to demonstrate the ability of their laser fluorescence apparatus to detect the presence of demineralisation at the periphery of an amalgam restoration. Following on from this pilot study, they subsequently compared the abilities of laser fluorescence and visual examination to detect marginal demineralisation and demonstrated superior results with laser fluorescence. Furthermore, over a subsequent 17 day remineralisation programme, they reported success in demonstrating longitudinal monitoring of

remineralisation of marginal lesions. The authors therefore suggested that LF may have a possible future role in secondary caries management.

In situ changes in mineralisation were demonstrated using laser fluorescence by Al-Khateeb et al., (1997b). Enamel samples were cut from extracted premolar teeth with the natural surface kept intact, and subjected to a pH-cycling system to produce subsurface demineralisation *in vitro*. The enamel blocks were then inserted into composite holders bonded to the buccal surfaces of both upper first molars of 12 human subjects. Various remineralisation protocols involving topical fluorides were then followed each lasting 5 weeks. Fluorescence radiance was measured before, during, and after *in vitro* demineralisation, and once a week during the *in situ* experimental periods. The enamel samples were then sectioned and analysed with TMR. At the end of the 5-week *in situ* periods, fluorescence radiance had been regained to a level of 80-100% of the value before *in vitro* demineralisation, indicating remineralisation of the enamel samples. There was a highly significant linear dependence ($p < 0.001$) between the final measurements obtained with the laser fluorescence method and the data obtained from TMR, $r = 0.76$. The authors concluded that with the sensitive laser fluorescence method it was possible to register the small changes in the enamel week by week during *in situ* remineralisation.

Al-Khateeb et al., (1998) have also used laser fluorescence to monitor remineralistaion in naturally occurring demineralised lesions. They reported longitudinal *in vivo* quantification of changes in incipient enamel lesions related to

fixed orthodontic appliances. Seven young patients with active caries lesions disclosed at removal of the orthodontic brackets and bands were enrolled in the study. The caries lesions were monitored with the quantitative laser fluorescence method after removal of the brackets and once a month thereafter. During the period of study, preventive measures such as dietary advice and use of fluoride containing toothpaste were taken. For each lesion, the three parameters lesion area (mm^2); mean fluorescence loss (%) over the lesion and maximum loss of fluorescence (%) in the lesion were measured. During a 1-year follow-up period, the areas of the lesions decreased and the enamel fluorescence lost was partly regained indicating that a remineralization process had occurred. From these results, it was concluded that quantitative laser fluorescence also seemed applicable for *in vivo* monitoring of mineral changes in incipient enamel lesions.

The majority of studies involving laser fluorescence detection of demineralisation have studied smooth surface lesions. However, Ferreira Zandoná et al., (1998a) reported their results of laser fluorescence in artificial occlusal fissures. This study also included a comparison of laser fluorescence with dye-enhanced laser fluorescence (DELF). DELF is discussed further in section 1.5.7.

The authors speculated that the morphology of the fissure and the presence of plaque and other debris in the fissure base may have an influence on the practicality of the technique in detecting occlusal caries. They created a model to simulate occlusal fissures by mounting polished blocks of bovine enamel in various configurations to

create different fissure morphologies. The fissures were exposed to a demineralisation protocol to create a variety of patterns of sound and demineralised fissure bases and walls. In addition, some of the fissures were inoculated with plaque samples to study the influence of this factor on the analysis.

One half of each group was examined with laser fluorescence (LF) and dye-enhanced LF (DELF); the other half was examined with LF, exposed to plaque, examined with LF and DELF, air-polished and examined with DELF. All images were scored twice as either carious, sound or undetermined by a group of 3 examiners. For fissures without plaque, the average sensitivity was significantly higher for DELF (0.76) than for LF (0.54). Likewise, the average specificity was significantly higher for DELF (0.64) than for LF (0.29). In the presence of plaque, sensitivity was higher for DELF (0.91) compared to LF (0.43); however, specificity was lower for DELF (0.05) compared to LF (0.55). In comparison to the plaque infested fissures, once the fissures were air-polished and then re-examined with DELF, sensitivity averaged slightly less (0.82) but specificity increased significantly (0.51). It was concluded that, in the absence of plaque, DELF was a better diagnostic tool than LF for detection of demineralization in artificial fissures. However, more important than the distinction between DELF and LF is the illustration of the potential influence of plaque, fissure morphology, debris, stain etc. in using laser fluorescence to detect occlusal caries.

In a subsequent study, Ferreira Zandoná et al., (1998b) reported the comparison of laser fluorescence and visual examination in detection of demineralisation in occlusal pits and fissures in extracted human teeth. The aim of this study was to compare the sensitivity and specificity of visual examinations, laser fluorescence (LF) and dye-enhanced LF (DELF) for detecting demineralization in occlusal pits and fissures. The actual presence of lesions was subsequently determined by histological examination. Independent clinical examiners visually graded three sites on occlusal surfaces of extracted, human premolars as sound or carious and also rated the colour of each graded site as: 0 = same as surrounding enamel; 1 = white; 2 = light brown, or 3 = brown/dark brown. An argon laser was used to illuminate the teeth for LF and DELF and the images were captured with a CCD camera and then analysed. DELF images were captured after the teeth had been exposed to 0.075% sodium fluorescein. Sections were then cut from each specimen and analysed by confocal laser microscopy and histology for the presence or absence of caries. Results showed that DELF was significantly more sensitive than LF and visual examination for detecting caries, but that there were no significant differences among the methods in specificity. When colour was used as an indication of caries in the visual examination, the diagnostic ability of this test was not different from LF. Results indicated that DELF was the best diagnostic tool and that visual examination and LF were equally effective as diagnostic methods, when colour of fissures was included as an indication of demineralization in the visual examination.

The detection of approximal caries by laser fluorescence has also been investigated by Eggertson et al., (1999). This *in vitro* study evaluated the use of laser fluorescence for the detection of early interproximal carious lesions and also whether the detection could be enhanced using a fluorescent dye. Direct visual examination was used for comparison. Eighty extracted teeth were utelised, arranged in 20 blocks and lined up in a simulated sextant situation. After surface preparation with a microabrasion kit, a subcontact window on half of the surfaces was exposed to Carbopol demineralising solution for 5 days. The teeth were remounted in stone and examined by three independent examiners. For LF and DELF an argon laser was used of wavelength 488 to 514 nm and viewed through glasses to exclude wavelength <520 nm. For DELF a sodium fluorescein dye (0.075%) was applied before examination. The approximal surfaces were scored for presence or absence of lesions and using staining and microscopy as the gold standard, sensitivities and specificities were calculated for each technique. Using this *in vitro* model DELF compared favourably with direct visual examination and LF in sensitivity, but specificity was better for DELF and visual examination than for LF.

González-Cabezas et al., (2000) investigated the measurement of early *dentine* lesions using quantitative light-induced fluorescence (QLF). The purpose of the study was to investigate the ability of the QLF system to detect and quantify incipient demineralisation in dentine. Extracted teeth were ground and polished to remove the enamel and the outer 1mm of dentine. A small area of each specimen was then demineralised for different time periods before QLF images of the artificially

created demineralised lesions were captured. Sectioning and analysis by microscopy was then undertaken to determine the depth of the lesions. Average change in fluorescence measured by QLF analysis was seen to differ significantly only for the group of lesions which had been demineralised for the longest time period where as the microscopic analysis of lesion depth indicated significant differences between all groups. The correlation of the two techniques was measured by Pearson's correlation coefficient as 0.64 which was highly significant. The authors therefore concluded that the QLF technique is a valid means of detecting and quantifying small carious lesions in dentine.

1.5.6 The Use of Dyes in Caries Detection

Dyes have been reported as being able to facilitate caries detection and the use of dyes in conjunction with light fluorescence for caries detection has also been reported (van de Rijke 1991). O'Brien et al., (1989) have described the use of an absorbing dye to enhance colour contrast between carious lesions and the surrounding tooth structure.

A variety of chemical compounds have been used to dye carious lesions. Procion dyes have been used to stain enamel lesions and although this technique results in an enhanced visualisation of the lesion, the staining is irreversible due to reaction of the dye with OH and NH_2 groups acting as a fixative (Lee et al., 1986). Brooke et al., (1972) have reported the successful use of Calcein to infiltrate enamel lesions *in vivo*

but reported that this dye complexes with calcium ions and may permanently bind to the lesion. Rawls and Owen (1978) also described a commercially available fluorescent dye, Zyglo ZL-22, to penetrate previously undetectable lesions which became visible under ultra-violet light. However, this dye was not suitable for intra-oral use and was only studied *in vitro*.

O'Brien et al., (1989) used the dye 10% Aqueous Brilliant Blue in conjunction with fiber optic transillumination and demonstrated improved detection of incipient carious lesions. The authors noted however, that the enhancement was only detectable by colourometric analysis of photographs and was not suggested as being applicable in a clinical technique.

Quantification of caries was attempted by van de Rijke and ten Bosch (1990) who described the use of the dye Fluorol 7GA in a study where they measured the fluorescence from stained lesions with a photo-diode. Subsequent microradiography of the lesions demonstrated a linear correlation between mineral loss and fluorescence intensity.

In another study by van de Rijke and co-workers, a fluorescent dye was applied to extracted premolars with either early artificial lesions or natural white-spot lesions (van de Rijke et al 1991). The teeth were placed in an approximal geometry, and with a specially designed fibre-optic probe the fluorescence of the dye was measured in the lesions. The mineral loss from the lesions was measured with microradiography

for the artificial lesions, and the optical caries monitor for the natural white-spot lesions. The correlation coefficient between corrected fluorescence and mineral loss was r = 0.86 and it was therefore concluded that measurement of dye uptake may be a very sensitive method to diagnose early approximal caries lesions and furthermore may enable quantification of these lesions.

Dyes for staining carious dentine have also been developed and Fusayama and Terachima (1972) described the successful selective staining of infected demineralised dentine using 0.5% basic fuchsin. Further studies by List et al., (1987), using this material as a guide to caries removal during cavity preparation, indicated that the dye was helpful in indicating caries that may otherwise be missed. Since the initial development, the fuchsin dye has been replaced due to concerns over the carcinogenic potential of fuchsin and clinical trials undertaken which have suggested the superiority of the acid red dye over clinical judgement alone in the removal of softened, infected dentine (Kidd et al., 1989). A commercially available product has been described by Fusayama (1988).

1.5.7 Dye Enhanced Fluorescence

Section 1.5.5 has discussed the ability of various laser fluorescence techniques to detect demineralisation of enamel and dentine both *in vitro* and *in vivo.* A number of the quoted papers also include some analysis of dye-enhanced laser fluorescence (Ferreira Zandoná et al., 1998a;1998b; Eggertson et al., 1999). It has been suggested

that the use of a fluorescent dye to penetrate the lesion may further enhance the detection of early carious leisons by QLF techniques (Hall et al., 1996)

A dye enhanced fluorescence technique for the quantification of demineralised root dentine was reported by van der Veen and ten Bosch (1996). The method was tested on 40 specimens of *in vitro* demineralised parts of human root dentine. Fluorescein sodium salt was used as a penetrating dye and the fluorescein sodium salt excited using light around 465 nm. The fluorescence signal around 527 nm measured for demineralised dentine was compared to the sound control dentine on the same root to obtain a fluorescence value. The values obtained for the specimens correlated linearly ($r = 0.91$) with mineral loss as measured by transverse microradiography. Therefore, it was concluded that this technique could potentially provide a good, non-destructive measure of the severity of *in vitro* demineralised human root dentine. The authors acknowledged that further work on natural lesions *in vivo* would be needed to investigate the applicability of the technique in a clinical situation.

Ando et al., (1997) undertook an *in vitro* study to explore the influence of a dye on the use of laser fluorescence in the detection of very early demineralization. The methods evaluated in this study were quantitative laser fluorescence (QLF) and an experimental dye-enhanced laser fluorescence (DELF) technique. Prepared and polished bovine enamel specimens were demineralised in a conventional lactic acid-Carbopol solution for varying periods of time between 0 and 24 h with an area of sound enamel retained on each specimen. The specimens were then analysed for the

presence and severity of enamel demineralization using QLF after which they were exposed to a selected dye (Pyrromethene 556) and similarly examined using DELF. The specimens were then sectioned and examined by conventional transverse microradiography and by confocal laser-scanning microscopy. Results were analysed statistically with sensitivity and specificity determined using sound enamel as the reference. The results indicated that QLF could detect demineralization which occurred as a result of 8 h exposure to the decalcification solution and was able to quantify changes in lesion severity associated with longer demineralization. While DELF was capable of detecting enamel demineralization after only 2 hours exposure to the decalcification solution, it was unable to quantify increasing amounts of demineralization associated with longer periods of exposure to the decalcification solution. These authors therefore concluded that while DELF was able to detect very early demineralization, only QLF was capable of detecting and quantifying changes in the extent of the decalcification occurring with demineralization periods up to 24 hours.

The use of the dye sodium fluoroscein 0.075% has been tested as an adjunct to laser fluorescence by Ferreira Zandona et al., (1998a, 1998b) and also by Eggertson et al., (1999). These papers report *in vitro* trials involving an artificial fissure model using bovine enamel; naturally occurring lesions on occlusal surfaces of extracted human teeth and artificially created lesions on approximal surfaces of extracted human teeth respectively. In each of these models, when histology was used as the gold standard for the presence of caries, dye enhanced laser fluorescence was shown to be a

superior technique for the detection of demineralisation than either visual examination or laser fluorescence without dye enhancement.

1.6 The Development of the DIAGNOdent Device

Recently, Hibst and Gall (1998) have described a new approach to fluorescence spectroscopy for caries detection. Previous research in laser fluorescence, as described in section 1.5.5, has concentrated on stimulation with ultra-violet, blue or green light with emission at a longer wavelength occurring in the visible spectrum for easier detection and analysis. Hibst and Gall (1998) however, demonstrated a better fluorescence of carious tooth structure when excited in the red and detected in the near infra-red portion of the spectrum. At these wavelengths, they demonstrated a much more intense fluorescence of carious tissue relative to sound tooth tissue. This is in contrast to the work of Sundström et al., (1985) who, following stimulation at shorter wavelengths described a more intense fluorescence from sound enamel relative to carious. At longer wavelengths, inducing relatively more fluorescence in carious tooth tissue, Hibst and Gall (1998) therefore described the possibility of 'optically probing' beneath sound surface enamel to detect caries in deeper layers.

Hibst and Gall (1998) also reported the results of excitation at these longer wavelengths and described the development of a clinically applicable device. They excited freshly extracted human teeth with and without carious lesions and extended the excitation into the red portion of the spectrum. It was noted that fluorescence decreased at the longer wavelengths but that the decrease was much more pronounced for sound compared to carious enamel or dentine i.e. there was a more intense fluorescence of carious relative to sound tooth tissue. At 638 to 655nm, the

intensity of fluorescence of caries was seen to exceed healthy tissue by more than one order of magnitude. It was suggested therefore that caries may be detected by fluorescence intensity rather than by analysing actual spectral differences between sound and carious tissue.

Hibst and Gall (1998) go on to describe the development of a clinically applicable system incorporating a diode laser as a light source. This represents the device now marketed by the KaVo dental company as the KaVo DIAGNOdent 2095.

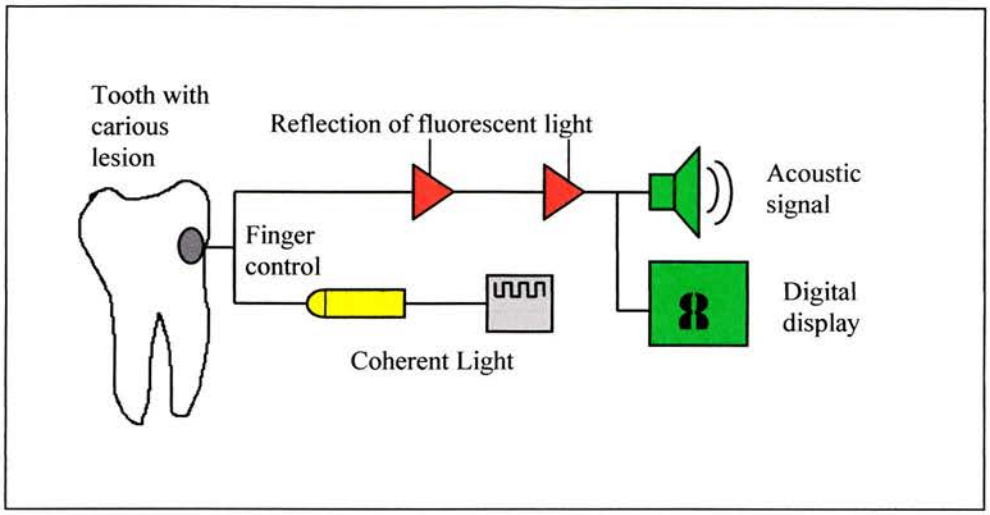
1.6.1 The DIAGNOdent Caries Detection System

The DIAGNOdent Caries Detection System (KaVo DIAGNOdent 2095, KaVo Dental GmbH, Biberach, Germany) consists of a base unit containing the laser source and electronics; a fibre optic lead; and interchangeable probe tips for delivery of the light to the tooth surface. The whole system is packaged as a portable battery powered unit measuring 150mm x 110mm x 120mm and weighing 600 grammes (figure 1.3).

Figure 1.3 The DIAGNOdent Device

The technical specification of the DIAGNOdent device describes a Class 2 laser of power less than 1 mW and of wavelength 655nm. The light source is transported to the angulated tip within a central optical fibre. Around this central fibre, additional fibres are concentrically arranged to collect the fluorescent light from the tooth surface. Reflected and ambient light is eliminated by a long pass filter allowing only the fluorescent light to be measured by a photo diode detector. The detected light is quantified and displayed on an LED on the base unit as a numerical value between -9 and +99. Two LED indicators display the 'moment' or real time value and the 'peak' or maximum recorded value. The principle of the DIAGNOdent's operation is represented in figure 1.4.

Figure 1.4 Diagrammatic Representation of the Operation of the DIAGNOdent.



Two designs of interchangeable, autoclavable probe tip are included. A narrower 'A' tip is designed for use in the fissures of the occlusal surface and a broader, flatter 'B' tip is used for flat surfaces (figure 1.5).

Figure 1.5 Interchangeable Probe Tips for the DIAGNOdent

1.6.2 Clinical Use of the DIAGNOdent Device

Included with the device is a material of specifically known fluorescence which is intended for calibration of the instrument. Prior to use, the probe is held in contact with this reference material and the device activated according to the manufacturers instructions. The subsequent displayed value should be within 3 units of the accepted fluorescence value of the calibration material to ensure accurate performance.

Tooth specimens may then be assessed using the hand held probe tip. The manufacturers stress the importance of a clean, dry tooth surface prior to examination. In the first step of 'adaptation', the instrument is given a reference value for sound tooth tissue by holding the probe in intimate contact with an area of sound tissue (conventionally a cusp tip) and activating the appropriate controls according to the instructions. The probe is then placed against the tooth surface over the suspected caries or area of tooth under study and the fluorescence value for that site is viewed on the LED display. Both current and maximum values are displayed. Conventionally values up to '9' are considered within normal limits for sound tooth tissue and those exceeding this may be indicative of caries. The value '0' may be displayed for an apparently healthy spot on the surface and values up to '-5' may be obtained if even 'healthier' tissue are encountered. In this situation, the adaptation procedure should be repeated in that area.

1.7 The Origin of Fluorescence in Teeth

In 1991 researchers attempted to investigate in greater detail the constituents of enamel which are responsible for the fluorescence phenomenon (Hafstrom-Björkman et al., 1991). They studied the fluorescence emission pattern in dissolved human enamel and in different molecular weight fractions obtained using the techniques gel chromatography and dialysis followed by ultrafiltration. For comparison, solutions of synthetic hydroxyapatite and bovine enamel were also analysed. When the entire emission and excitation spectra of the corresponding excitation and emission wavelengths in the solutions of human enamel and bovine enamel were compared, no distinct differences were found between the solutions. With excitation at 375 nm, emission peaks were found at 460 and 560 nm, indicating the presence in human enamel solution of two different chromophores, unevenly distributed over the molecular weight fractions. The authors stated that the 460-nm and the lower 560-nm fluorescence peaks appeared to be derived from both organic and inorganic components.

Hibst and Paulus (1999) also carried out some research in an attempt to identify the origin of the fluorescence. A variety of samples were investigated by emission spectroscopy using a 655nm laser diode for excitation. Little or no fluorescence was found from healthy gingiva, blood, synthetic hydroxyapatite (HA). Staining of HA with a solution of black tea produced a fluorescent emission and bacterial colonies grown from caries swabs also showed some fluorescence. Natural teeth showed a

varying fluorescence depending on their colour intensity and it was noted that this was not altered by artificially decalcifying the enamel. The authors state that analysis of the emission spectra of these materials supports the hypothesis that fluorescence of sound teeth in vivo may in fact result from the absorption of organic molecules onto the inorganic crystal matrix. In addition, the increase in fluorescence seen as a result of the carious process may result from bacterial metabolites rather than mineral loss per se.

In follow up work, Hibst and Paulus (2000) attempted to identify the specific fluorescing molecules in carious teeth. They used high pressure liquid chromatography to analyse caries from freshly extracted teeth which had shown fluorescence when stimulated by 655nm laser diode *in situ*. Extracted porphyrins, in particular proto-porphyrin IX were identified as fluorescing molecules although this was successfully demonstrated by 406nm excitation and not 655nm. Analysis of solutions of porphyrins with the DIAGNOdent revealed a linear relationship between the concentration of porphyrin and the DIAGNOdent value. However, the fluorescence signal from these molecules, as detected by the DIAGNOdent device, was found to be enhanced considerably when solutions were mixed with synthetic HA or dried on tooth surfaces and the authors speculated that this may be due to a light scattering effect. They concluded that further work is required to determine if any other molecules contribute to the fluorescence.

1.8 Evaluation of the DIAGNOdent Device

A number of reported trials and experiments have attempted to assess the ability of the commercially produced DIAGNOdent device to accurately detect, monitor and quantify the carious process. Some studies have evaluated the validity and repeatability of DIAGNOdent analysis of tooth samples whilst others have compared the DIAGNOdent to a variety of other methods of caries diagnosis with results often expressed as a measure of the inter-method agreement. Studies comparing the results of DIAGNOdent assessment of caries to the histological status have been carried out and assessment of the ability of the device to accurately quantify mineralisation changes using microradiography as a gold standard have also been reported. The specificity and sensitivity of the DIAGNOdent at varying degrees of carious status have been frequently reported on the basis of the results of the 'gold standard' test.

1.8.1 *In Vitro* Trials using Natural Caries

In an early trial to investigate the DIAGNOdent system, Lussi et al., (1998a) reported on the 'Performance of a Laser Fluorescence System for Detection of Occlusal Caries'. In this study, 91 extracted molar teeth were thoroughly cleaned using NaOCl and pumice. DIAGNOdent analysis was carried out on both wet and dry occlusal surfaces prior to sectioning and histological examination of each tooth to determine the true extent of the carious lesion. On this basis, the best cut-off limits for this device for caries at the D2 level (extending to greater than half enamel thickness) and

D3 level (caries extending into dentine) were determined. Using these limits, sensitivity and specificity were calculated. On wet occlusal surfaces at the D2 level, sensitivity was 84% and specificity 80%. At the D3 level sensitivity was 64% and specificity 86%. Drying the occlusal surface prior to analysis had some effect and resulted in sensitivity and specificity at the D2 level of 79% and 74% respectively. At the D3 level the values for sensitivity and specificity were 76% and 79%. These results were sufficient for the authors to consider the DIAGNOdent system a useful tool in detecting occlusal caries. However, the adopted cut-off levels as well as the effect of other factors such as presence of plaque and fissure morphology were considered as uncertainties.

Lussi et al., (1998b) also investigated the reproducibility of the DIAGNOdent system in the detection of occlusal caries *in vitro*. 83 extracted molars were thoroughly cleaned and mounted in plaster blocks before assessment with the DIAGNOdent by 11 different dentists. Each dentist examined every occlusal surface twice and analysis of results indicated a very good reproducibility between the 2 readings. It was concluded that this high reproducibility suggests the potential for the use of the DIAGNOdent in longitudinal monitoring of carious lesions.

Klimm et al., (1999) compared the accuracy and reproducibility of visual inspection and laser fluorescence using the DIAGNOdent for diagnosing early occlusal caries *in vitro*. They also included a radiographic examination of the teeth for comparison and furthermore carried out histological validation of the carious status of the sites under

examination. Using histology as the gold standard these authors also reported Spearman correlation coefficients, sensitivities and specificities for each method but concluded only that laser fluorescence was able to increase the accuracy of early occlusal caries diagnosis rather than stating which diagnostic technique was superior.

Shi et al., (2000a) studied the DIAGNOdent with respect to reproducibility and validity and compared it with radiography regarding accuracy in the detection of occlusal caries. Seventy-six extracted premolar and molar teeth were measured twice, at an interval of 2 weeks, with DIAGNOdent under both wet and dry conditions. In addition, conventional film radiographs of the teeth were exposed. Microradiographic analysis of sections of the teeth served as the gold standard. Under both wet and dry conditions, the reproducibility of the DIAGNOdent method was considered to be excellent: $r = 0.97$ and 0.96, respectively. In addition, the diagnostic accuracy of DIAGNOdent was significantly better than that of radiography. Therefore, in this *in vitro* study of detection of occlusal caries, the diagnostic performance of the DIAGNOdent method was superior to that of radiography.

Other workers have also compared DIAGNOdent analysis with radiography for approximal caries detection. Longbottom et al., (1999a) set extracted premolar and molar teeth into quadrant blocks and exposed bitewing radiographs of the simulated 'quadrant'. In addition, the DIAGNOdent was used to examine the buccal and lingual embrasures of the 'quadrants'. Direct visual examination of the approximal surface was used as the gold standard for presence of caries. Sensitivity and specificity

values for caries in enamel with no break in surface integrity (D1) and caries in enamel with a break in the surface integrity or with shadowing indicative of dentine caries (D3) were calculated for both diagnostic techniques. At the D1 level radiography produced sensitivity and specificity scores of 35% and 95% respectively and DIAGNOdent analysis produced scores of 55% and 90%. At D3 level, radiography produced sensitivity and specificity scores of 64% and 96% respectively and DIAGNOdent analysis produced scores of 76% and 92%. The authors concluded that this *in vitro* model suggested that DIAGNOdent analysis is comparable to bite-wing radiography for approximal caries detection.

Lussi et al., (1999a) carried out an *in vitro* study designed to assess the validity of the DIAGNOdent device by comparing the values obtained in assessment of carious lesions with those of an electrical caries detection device. In addition, the study was designed to assess the reproducibility of DIAGNOdent readings. 105 extracted teeth with macroscopically intact occlusal surfaces were measured by a single examiner, using both the DIAGNOdent (on both moist and dried teeth) and an Electronic Caries Monitor (ECM). The teeth were subsequently examined histologically to determine the specificity and sensitivity at the D2 (caries extending through more than half of the enamel thickness) and D3 (caries involving dentine) levels. The values obtained for the DIAGNOdent ranged from 0.72 to 0.87 (specificity) and 0.76 to 0.87 (sensitivity). In comparison, those for the ECM ranged from 0.64 to 0.78 (specificity), 0. 87 to 0.92 (sensitivity). In addition, to determine intra- and inter-examiner reproducibility of the DIAGNOdent, 11 dentists recorded two different

measurements at the same site on a separate set of 83 extracted molar teeth, and these were compared at the D2 and D3 levels. The Spearman correlation value for intra-examiner reproducibility was 0.97. For inter-examiner reproducibility, the average kappa values were 0.65 at the D2 level and 0.73 at the D3 level with a Spearman correlation of 0.84. It was concluded that for occlusal caries the DIAGNOdent device has a higher diagnostic validity than the ECM, and *in vitro*, measurements using the device are highly reproducible. These authors concluded that the DIAGNOdent device could therefore be a valuable tool for the longitudinal monitoring of caries and also for assessing the outcome of preventive interventions.

A comparison of several methods for detection of occlusal caries was undertaken recently by Côrtes and Ellwood (2000). A single examiner assessed 152 occlusal surfaces of extracted molar teeth by visual inspection, fibre-optic transillumination (FOTI), combined FOTI/visual inspection, DIAGNOdent and an electrical caries meter (ECM). Visual and FOTI assessments were recorded simply as sound, stained, white spot, brown spot and dentinal lesions. Histological sections were made to validate the assessments and to determine the extent of lesions. Sensitivity and specificity was reported for all techniques for both enamel and dentinal lesions. The conclusions of this study were limited to stating that all methods showed a 'good' performance at detecting carious lesions in both enamel and dentine. In addition, the effect of staining was seen to influence the diagnostic ability of each technique and performance was improved by excluding assessment of stained lesions.

Tranæus et al., (2000) have also compared the DIAGNOdent and another QLF device for detection and quantification of caries on smooth surfaces. Non-cavitated smooth surface carious lesions from extracted premolar teeth were analysed by the two methods. In addition both histological and TMR analyses were carried out on sections from each lesion to determine lesion depth which served as the gold standard. Pearson's correlation coefficients between the gold standard and the two test methods were calculated and found to be 0.85 for QLF and 0.73 for DIAGNOdent. The sensitivity and specificity of caries diagnosis for the two test methods were not significantly different. However, the authors conluded that QLF was superior to DIAGNOdent analysis for smooth surface caries diagnosis.

Smooth surface lesions were also quantified using the DIAGNOdent by Shi et al., (2000b). 80 smooth surface lesions on extracted teeth were analysed with the DIAGNOdent by 2 separate examiners. Lesions were subsequently studied histologically and by TMR. Lesion depth as measured by histology and DIAGNOdent readings were compared and found to have a Pearson's correlation coefficient of 0.72-0.75 depending on the storage medium of the tooth samples. The correlation of DIAGNOdent to mineral loss as measured by TMR was much poorer with a Pearson's correlation coefficient of 0.58.

1.8.2 *In Vitro* Trials using Artificial Caries

Ando et al., (1999) carried out an *in vitro* comparison of three different types of quantitative light induced fluorescence (QLF). Enamel specimens prepared from extracted human teeth were demineralised for a specified time up to 120 hours. The specimens were illuminated by 488nm argon laser (QLF1), 290-450nm violet-blue light (QLF2) and images captured with a digital camera. Analysis of the demineralised specimens in relation to an undemineralised portion of each specimen was carried out using proprietary software designed for this purpose. In addition, DIAGNOdent analysis of each specimen was performed. A section from each specimen was also analysed by transverse micro-radiography (TMR) to serve as the gold standard for the quantification of mineral loss in the specimen. The correlations of mineral loss as measured by TMR to QLF1 and QLF2 were similar at 0.82 and 0.83 respectively and were better than the correlation to DIAGNOdent which was 0.58. For lesion depth, correlations for QLF1, QLF2 and DIAGNOdent were 0.83, 0.86 and 0.59 respectively. These results suggested that either of the argon laser or visible violet-blue light methods is a superior method of quantifying mineral loss than DIAGNOdent analysis.

An *in vitro* study to investigate the relationship between DIAGNOdent readings and the enamel caries process was carried out by Iijima and Takagi (2000). Enamel specimens were demineralised at pH 5 for 3, 6 or 9 days and then remineralised at pH 7 for 9, 18 or 27 days. After the demineralisation period and also at the end of

remineralisation, 100μ sections from the lesions were analysed by TMR. The DIAGNOdent readings were, in all cases, significantly raised following demineralisation and significantly reduced following remineralisation although the maximum DIAGNOdent reading was not simply linearly related to mineral loss or lesion depth as measured by TMR. This apparent lack of relationship between DIAGNOdent value and mineral loss is consistent with the findings of Ando et al., (1999).

1.8.3 *In Vivo* Trials

Reich et al., (1998) carried out a simple *in vivo* assessment of the clinical accuracy of the DIAGNOdent. 24 patients were examined clinically, radiographically and with the DIAGNOdent. 55 posterior teeth were diagnosed as carious with the DIAGNOdent but were clinically doubtful. These fissures were carefully opened up with a diamond bur to determine the carious state of the fissure and the extent of the caries was noted as either in enamel only or into dentine was noted. All of the 55 clinically doubtful fissures were subsequently found to be carious. The DIAGNOdent assessment of those fissures which were confined to enamel (mean value 29, range 15-47) was significantly different from those which were found to extend into dentine (mean value 56, range 20-99). These data are claimed to demonstrate the ability of the DIAGNOdent device to detect caries *in vivo* when direct clinical fissure exploration is used as the gold standard for presence or absence of caries. Furthermore, this seems to suggest a correlation between the DIAGNOdent value and

the clinical extent of the caries. This is in contrast to the apparent lack of relationship observed between DIAGNOdent value and degree of demineralisation (Ando et al., 1999; Iijima and Takagi 2000).

In a similar study, Verdonschot et al., (1999) published their assessment of 'The *in vivo* Performance of a Laser Fluorescence Device Compared to Visual Inspection in Occlusal Caries Diagnosis'. In this study, 41 permanent teeth scheduled for fissure sealents were examined by visual inspection by 2 different examiners and were scored 0-3 depending on the extent of the carious lesion (0=sound, 1=enamel only, 2= less than 1mm into dentine, 3= more than 1mm into dentine). DIAGNOdent assessment of each tooth was carried out and the highest score noted. The fissures were explored with a diamond bur and the extent of the caries agreed by different examiners. Inter-examiner reproducibility was assessed by correlation coefficient and was higher for DIAGNOdent (0.93) than for visual inspection (0.79). The validity of the 2 methods was expressed as sensitivity and specificity and it was found that visual inspection (sensitivity 0.50, specificity 0.86) was superior to DIAGNOdent assessment (sensitivity 0.17, specificity 0.83). However, this abstract does not discuss the degree of correlation between DIAGNOdent value and clinical extent of the caries determined by exploration.

Reich et al., (1999) conducted another *in vivo* comparison of the DIAGNOdent with traditional clinical diagnosis by visual inspection. This study also attempted to study the values recorded by the laser fluorescence device in relation to the clinical stage of

the carious lesions under study. In 40 patients, 50 carious lesions scheduled for restoration were graded clinically as enamel caries, dentine caries or caries profunda. A DIAGNOdent analysis of each lesion was also carried out. The lesions were then excavated to validate the clinical diagnosis and the prepared cavities once again examined with the DIAGNOdent to assess the residual dentine for caries. In 9 out of 50 lesions the pre-excavation clinical grading differed from the validated diagnosis by one grade. The mean DIAGNOdent value differed significantly between the 3 gradings of caries with clinically more advanced lesions scoring higher. After caries removal, the DIAGNOdent was still able to detect an increased reading from the cavity in comparison to surrounding sound tooth structure and the authors propose the use of the DIAGNOdent device as a means of assessing the complete removal of caries during cavity preparation. However, the potential of fluorescence of the pulp in deep lesions is considered.

Lussi et al., (1999b) compared the performance of the DIAGNOdent with both visual inspection and bitewing radiography and also defined what they considered to be useful cut-off limits for diagnosis with the device. 156 teeth in 110 patients were examined by 6 different dentists using visual inspection, bitewing radiographs and the DIAGNOdent. The dentist's clinical judgement provided the gold standard for carious status. When clinically indicated, the lesions under examination were opened up and the extent of the caries was noted. Comparisons were made between the DIAGNOdent value and the clinical and radiographic extent of the caries for each lesion. On this basis cut-off limits for DIAGNOdent values were determined as 0-15

for sound enamel, 16-25 for caries confined to enamel and 26 and above for dentinal caries. From this the authors went on to suggest guidelines for clinical use of the DIAGNOdent with values 0-15 requiring no active care, values 16-30 indicating preventive or operative care depending on caries risk and recall interval and values 31 and above definitely indicating operative intervention for that lesion. However, the basis on which this judgement is made is not clear form the published data and it seems simply that an arbitrary consensus on thresholds was reached on the basis of the clinical specimens under study.

Longbottom et al., (1999b) carried out an *in vivo* assessment of the sensitivity and specificity of the DIAGNOdent using histology as the gold standard. 36 non-cavitated premolar and molar teeth were scheduled for extraction for orthodontic purposes. A DIAGNOdent analysis of the occlusal surface of each tooth was performed both before and after cleaning with a Prophyjet device. Following extraction, teeth were sectioned and stained and examined microscopically at 3.2x and 6.4x magnification. Caries was scored as 0=sound; D1=caries in outer half of enamel; D2=caries in inner half of enamel; D3=caries in outer half of dentine and D4=caries in inner half of dentine. Using predetermined threshold values for the DIAGNOdent, sensitivity and specificity were calculated for D1, D2 and D3 carious lesions for both before and after cleaning with the Prophyjet device. The sensitivity of the DIAGNOdent was of the order of 80% but specificity values were lower and more variable. No discussion of the effect of cleaning the occlusal surface with the Prophyjet was made. It was concluded that the low specificities may suggest the need

for further investigation into the appropriateness of the threshold values used with the DIAGNOdent device.

Longbottom et al., (1998) have also compared the DIAGNOdent with an electronic caries monitor (ECM), (ECM, Sensor Technology, The Netherlands). The occlusal surfaces of 40 teeth scheduled for orthodontic extractions were examined visually, with DIAGNOdent and with ECM. No histology was reported for the specimens in this study and the gold standard for carious status was taken as the ECM readings. On this basis the sensitivity of the DIAGNOdent was found to be very high (100%) but its specificity was poor (27% for caries at D3 level). However, adjusting the DIAGNOdent threshold for dentinal caries from 11 to 18 resulted in sensitivity and specificity values of 90% and 82% respectively for caries at the D3 level. It was concluded that these values are similar to those reported for the ECM and therefore the two devices are comparable. In common with Longbottom et al., (1999b), this study also calls into question the arbitrary threshold values adopted when using the DIAGNOdent to clinically diagnose the extent of a lesion.

Another *in vivo* trial comparing the DIAGNOdent with visual examination for diagnosis of occlusal caries was carried out by Sheehy et al., (2000). 137 first permanent molars were examined in children using a visual ranking scoring system and the DIAGNOdent device. Although a Spearman correlation coefficient of 0.76 proved a significant correlation between the two methods, a number of lesions scoring the same on the visual scale were markedly different in respect of their

DIAGNOdent analyses. The authors suggest the inability of the DIAGNOdent to reliably measure lesion activity and the need for frequent recalibration are disadvantages of the system. It was concluded in this study that a lack of consensus on the diagnostic criteria for caries make comparison of different diagnostic tools difficult.

A different investigation into the efficacy of the DIAGNOdent was carried out by Lussi et al., (2000) who reported 'The Use of the DIAGNOdent During Cavity Preparation'. Reich et al., 1999 have previously discussed the possibility of the use of the DIAGNOdent assess caries removal during cavity preparation. This was further investigated by Lussi et al., (2000) who selected 64 carious cavities and explored them *in vivo* with three different examiners. Once cavity preparation had been completed and deemed to be caries free base on assessment with a sharp explorer, the cavity was further analysed using the DIAGNOdent device. Cavities extending into enamel only or into dentine showed no difference in DIAGNOdent value but those extending close to the pulp resulted in a significantly elevated DIAGNOdent value. This was considered to be a significant finding and may indicate the potential of fluorescence of the dental pulp to influence DIAGNOdent readings.

1.9 Summary, Aims and Objectives

1.9.1 Summary

Early diagnosis of caries is essential if disease progression is to be addressed. This literature review has shown that existing techniques for detection of caries have several disadvantages and that most techniques lack the ability to quantify the extent of a carious lesion. Alternative techniques are under development and much research has been carried out on methods which aim to satisfy the goals of early, accurate diagnosis along with some quantitative ability. The majority of the reported alternative diagnostic techniques are currently limited to experimental use and few have been commercially developed to provide devices applicable to routine clinical practice. However, some recently marketed devices have shown promise although there is still a lack of consensus as to their practical clinical value and traditional techniques remain the mainstay of caries diagnosis for the majority of clinicians. Therefore, further evaluation of such devices is desirable to learn more about their diagnostic ability, their limitations and their value in everyday practice.

The DIAGNOdent Caries Detection System is one device which has been commercially developed and has been introduced for clinical use in Europe and the United States. A variety of trials both *in vitro* and *in vivo* have been undertaken to evaluate its potential. However, a number of uncertainties remain over this device's

application to clinical practice and further study is needed before DIAGNOdent analysis can be confidently accepted as reflecting the true carious status of a tooth.

1.9.2 Aims and Objectives

Caries detection by the DIAGNOdent is claimed to be achieved by virtue of fluorescence from within the carious lesion. Although promotional literature from KaVo presents a series of studies designed to assess the ability of the device to detect caries *in vivo* or in extracted teeth, there are no data presented to demonstrate the ability of this device to quantify the amount of mineral lost as a result of demineralisation attack. As a result, is not clear whether the device is able to detect demineralisation *per se* or is simply detecting fluorescence from proteins within the lesion. Such fluorescent proteins are often found in naturally occurring carious lesions but would probably not be apparent in most laboratory-created lesions. Furthermore, inaccuracy due to the presence of other exogenous fluorophores within the lesion seems a possibility (Hibst and Paulus 2000).

The objective of this research was therefore to investigate the efficacy of the DIAGNOdent Caries Detection System. To achieve this objective, the specific aims of the investigation were:

1. To establish the ability of the DIAGNOdent system to detect demineralisation in artificially created carious lesions.

2. To examine its ability to assess demineralisation quantitatively.

3. To carry out a comparison with another optical caries detection system based on fluorescence (QLF \ clin Optical Measuring System).

4. To investigate the potential for influence from other fluorescent molecules such as exogenous staining materials.

5. To assess the ability of the DIAGNOdent to detect residual caries at the margins of restorations.

6. To examine, in greater detail, the phenomenon of fluorescence from carious lesions by investigating the pattern of fluorescence and relating this to the pattern of mineral loss within the lesion.

1.9.3 Null Hypothesis (No.)

The Null Hypothesis (No.) is that the DIAGNOdent Caries Detection System is unable to detect or quantify demineralisation.

CHAPTER 2

DETECTION OF DEMINERALISATION USING TWO FLUORESCENCE BASED CARIES DETECTION SYSTEMS

2.1 Introduction

The exposure of a tooth specimen to a demineralising system may be expected to cause demineralisation of the enamel of that tooth. Furthermore, the length of exposure may also be expected to influence the extent of mineral loss, with longer exposure resulting in a greater degree of demineralisation. Methods of assessment of demineralisation have been described in section 1.4 and the problems associated with the need for sample destruction discussed. Potential advantages of the DIAGNOdent in quantifying mineral loss are the conservation of the specimen under test and also its suitability for use *in vivo*.

It was proposed to assess the ability of the DIAGNOdent caries detection system to detect the demineralisation in laboratory produced artificial caries as well as its ability to quantify this by distinguishing between different periods of demineralisation of the lesions. In addition, to allow comparison, it was proposed to assess the same specimens using another fluorescence based caries detection system, the QLF-clin system.

2.2 Materials and Methods

2.2.1 Specimen Collection and Preparation

Extracted human teeth were collected on a daily basis and stored until use in 0.12% thymol solution. From this pool, 110 teeth were selected for large, intact buccal surfaces. Selected teeth were cleaned with a rotating bristle brush and oil free prophylaxis paste (SS White Prophylaxis Paste, SS White Manufacturing, Gloucester, England) to remove the salivary pellicle. Any existing restorations, along with residual or underlying caries were removed using a dental handpiece and burs. Teeth were then bisected bucco-lingually using a cutting machine with a diamond edged circular saw with water coolant (Labcut 1010, Agar Scientific Ltd, D.R. Bennett Ltd, Leceister, England) (figures 2.1-2.4). This yielded a total of 110 paired tooth samples.

Fig. 2.1 Extracted Molar Tooth Selected for Sectioning

Fig. 2.2 Labcut 1010 Sectioning Machine

Fig. 2.3 Tooth Mounted Ready for Sectioning

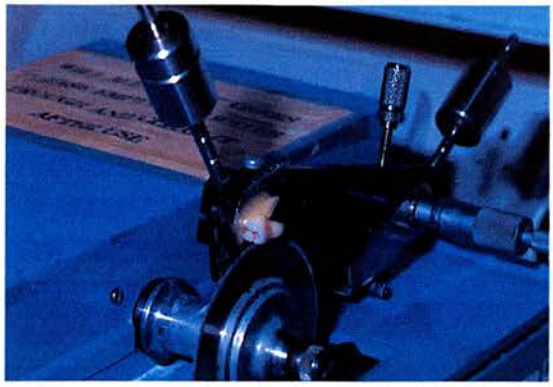


Fig. 2.4 Sectioned Tooth Yielding Paired Specimens

The paired tooth samples were numbered 1 to 110 and each half of the pair labelled 'A' or 'B'. This marking was effected in pencil onto residual root cementum which provided a durable mark. Sample pairs were then stored together in appropriately numbered individual sample bottles containing 0.12% thymol solution.

The 'A' sample of each pair was subjected to a protocol of surface abrasion. Approximately 300μm of surface enamel was removed from the tooth's buccal surface using a dental handpiece and diamond burs. Standardisation of this procedure was effected by the use of a combination of a depth gauged veneer bur (Komet, Gebr. Brasseler GmbH & Co.KG, 32631 Lemgo, Germany) followed by a 25μm grit high speed finishing bur (Komet, Gebr. Brasseler GmbH & Co.KG, 32631 Lemgo, Germany) to remove the residual enamel ridges and restore a smooth surface (figures 2.5 & 2.6).

Fig. 2.5 Burs for Abrasion of Enamel Surface

Fig. 2.6 Specimen Following 300μm Reduction of Buccal Surface

2.2.2 Production of Bare Enamel Windows

Each specimen was then painted with red nail varnish (Max Factor Diamond Hard Firebrand, Procter and Gamble, Weybridge, England) to cover its entire surface except for a window of approximately 3mm by 1mm (as measured using Vernier calipers) on the buccal surface (figure 2.7). Two coats of varnish were applied to each tooth half and the varnish allowed to dry for 24 hours prior to returning the specimens into screw-cap sample bottles containing distilled water.

Figure 2.7 Bare Enamel Window

2.2.3 Preparation of Demineralising Solutions

Two demineralising solutions were prepared for lesion creation in this study; 'Carbopol' solution has been described by White (1987) and 'Glasgow' solution has been reported by Damato et al., (1990). These are described in sections 1.3.5 and 1.3.6 respectively.

2.2.3.1 Preparation of 'Carbopol' Demineralising Solution

'Carbopol' / lactic acid gels were prepared from stock solutions of 1% polyacrylic acid and 1.0 mol/litre lactic acid respectively.

A. Preparation of Stock Solutions

The stock 1% polyacrylic acid solution was prepared by adding 10g of 'Carbopol' powder to a 1 litre beaker with approximately 600ml of distilled water. The resulting solution was stirred with a magnetic stirrer at room temperature for 2-4 hours until the 'Carbopol' was completely dissolved. With continued stirring, 10 molar Sodium Hydroxide was added and the pH of the solution adjusted to a value of 4. The pH was confirmed using a combination glass electrode (Meltron 620 pH meter). The resulting solution was transferred to a 1 litre volumetric flask and diluted to the mark.

The stock lactic acid solution was produced from reagent grade lactic acid and was steam distilled prior to use. This was produced by taking 74.5 ml of manufacturers lactic acid which had been heated at 90 degrees centigrade for 8 hours and making it up to 1 litre.

Finally a 1 molar sodium hydroxide solution was prepared. This was carried out by the slow addition of 4g of sodium hydroxide pellets into 100ml of distilled water. The solution was allowed to cool prior to decanting into a plastic bottle. The sodium hydroxide solution was made as close as possible to the time of use as carbon dioxide from the atmosphere is absorbed into the solution forming a weak solution of carbonic acid resulting in a neutralisation reaction with the formation of an alkali salt and a corresponding decrease in basicity. It should be noted that the heat of solution for sodium hydroxide is exothermic in nature, therefore during the addition phase of the sodium hydroxide pellets a substantial amount of heat is liberated resulting in a baseline increase in temperature.

B. Preparation of 'Carbopol' Demineralising Gel

'Carbopol' / lactate gels were prepared by the appropriate dilution of stock reagent solutions. The pH of the solution was adjusted by the dropwise addition of the 1.0 mol/l NaOH stock solution and was monitored using a combination glass electrode (Meltron 620 pH meter).

Saturated (with respect to hydroxyapatite) 'Carbopol' / lactate gel was first prepared by adding 2 grams of synthetic calcium hydroxyapatite to 1 litre of solution containing; 100ml of stock lactic acid solution and 200 ml of stock 'Carbopol' solution. This solution was adjusted to a pH of 5 by the dropwise addition of 2 molar hydrochloric acid solution. The solution was then allowed to reach equilibrium. This was indicated once the pH of the solution reached a value of 4.8 with a drift of less than 0.1 unit per hour. The solution was then filtered through filter paper (Whatman No.42, W & R Balston, England) and the supernatant collected. This was labelled 'Solution A'.

An unsaturated 'Carbopol' / lactate gel was then prepared in the same way as above but without the addition of the hydroxyapatite. In addition, 1ml of 10ppm fluoride was added and the pH was finally adjusted to a value of 5 with 1 molar sodium hydroxide. This was labelled 'Solution B'

A partially saturated demineralising solution was then prepared by adding together equal volumes of solutions A and B. This was stirred with a magnetic stirrer and readjusted to pH 5.0 by the dropwise addition of sodium hydroxide. This produced the final demineralising solution containing 50% saturated hydroxyapatite in 0.1 molar lactic acid containing 0.01 ppm fluoride at pH 5.

2.2.3.2 Preparation of 'Glasgow' Demineralising Solution

1 litre of 'Glasgow' demineralisation solution was prepared as follows:

0.222g of 2mM anhydrous calcium chloride and 0.224g of 2mM anhydrous sodium dihydrogen orthophosphate were placed in a beaker and 800ml of distilled water added. This was dissolved with the aid of a magnetic stirrer. 50 mM Acetic acid was added to the solution and the pH adjusted to 4.55 with the addition of 0.1 molar sodium hydroxide solution.

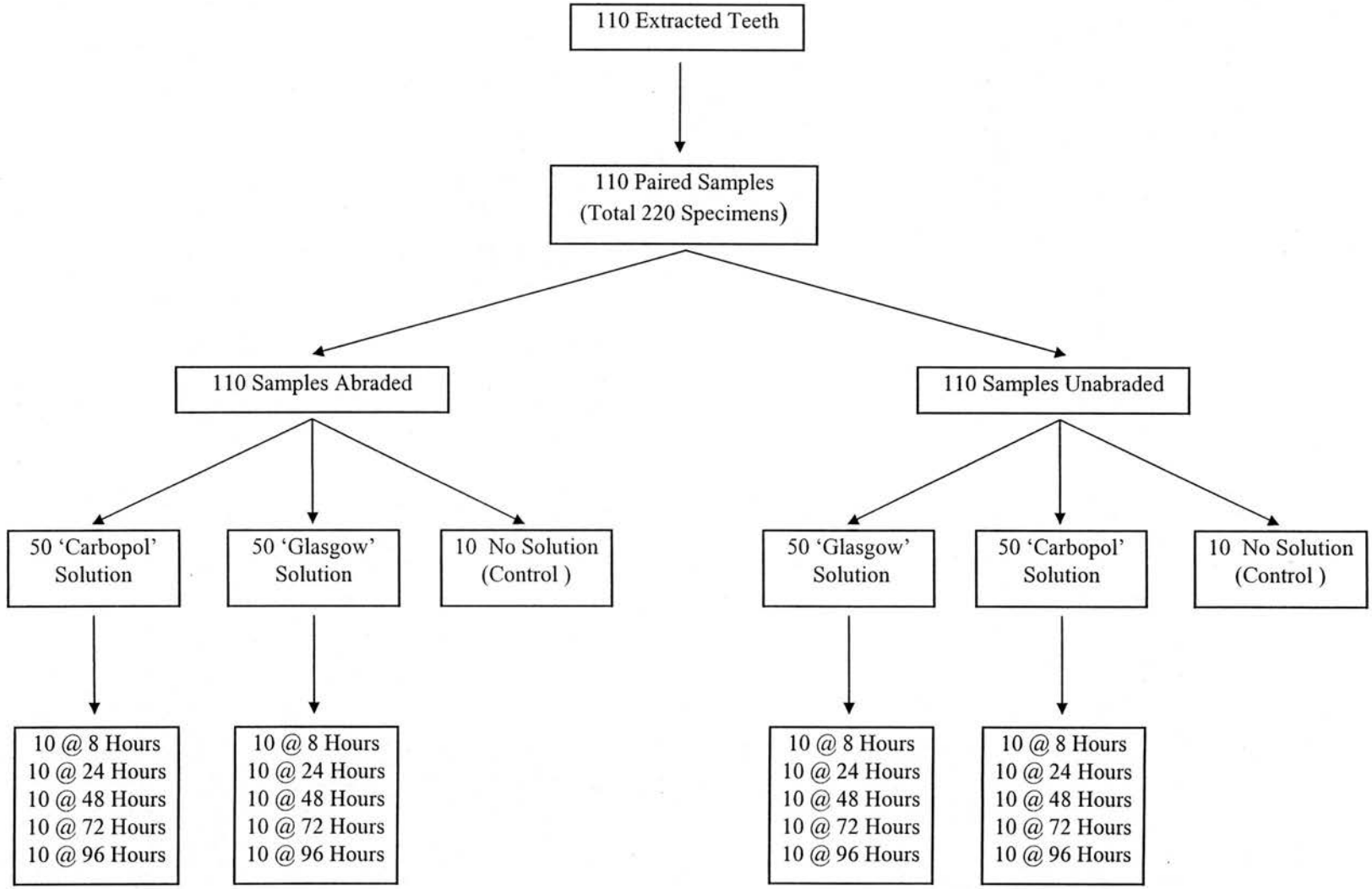
The solution was cooled and decanted into a volumetric flask. The beaker was rinsed with distilled water and added to the contents of the flask. To this flask, 1ml of 10ppm fluoride per litre of solution was added resulting in a final concentration of 0.01ppm fluoride. The solution was then made up to the mark and decanted into a plastic container for storage at 4 degrees centigrade until required for use.

2.2.4 Enamel Demineralisation

Each sample pair was subjected to one of the two demineralising solutions in a single sample bottle containing 25 ml of the solution. Samples 1 to 50 were exposed to the 'Glasgow' demineralisation solution and samples 51 to 100 subjected to the 'Carbopol' solution. Each of these two groups of samples was subdivided into 5 groups of 10 and each group exposed to the demineralisation solutions for one of 5

different time periods. The 5 demineralisation time periods chosen were 8, 24, 48, 72 and 96 hours. Those samples placed in the 'Glasgow' demineralisation solution had their demineralisation solutions renewed every 24 hours. The teeth subjected to the 'Carbopol' solution were stored at 37 degrees centigrade for the duration of the demineralisation. The remaining 10 teeth were used for the purpose of control and were not subjected to demineralisation. The experimental design and allocation of specimens to each lesion preparation protocol is summarised in figure 2.8.

Figure 2.8 Allocation of Specimens to Different Lesion Preparation Protocols



2.2.5 Varnish Removal

Following demineralisation, specimen pairs were washed thoroughly with water and were stored in a humid atmosphere generated by a cotton pledget bathed in thymol within individual sample bottles (figure 2.9). Varnish removal was carried out manually in a fume cupboard using acetone baths and a small brush. This was followed by an ethanol wash before returning samples to their bottles to be stored for analysis. A completed specimen with visible artificial lesion is illustrated in figure 2.10

Fig. 2.9 Demineralised Specimen Prior to Varnish Removal

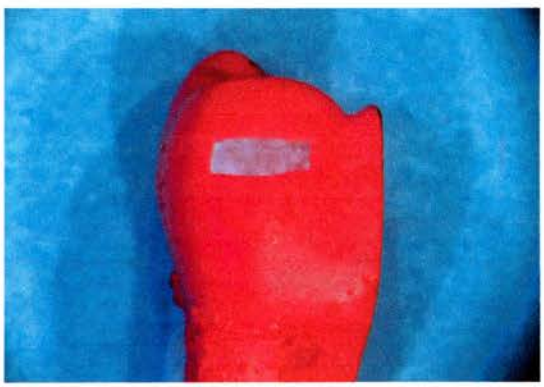


Fig. 2.10 Specimen with Artificial Lesion following Varnish Removal

2.2.6 Randomisation

In order to help blind the subsequent analysis, random number generation software was used to allocate each of the 110 paired samples a new identification number between 1 and 1000. In this way a total of 220 individual samples were created and placed for storage into appropriately labelled individual containers.

2.2.7 Analysis of Demineralised Lesions

Following the demineralisation process, the resultant artificial carious lesions were analysed using the two caries detection systems DIAGNOdent and QLF \ clin.

2.2.7.1 DIAGNOdent Analysis

Prior to analysis, the DIAGNOdent device was calibrated according to the manufacturers instructions using the supplied reference material of known fluorescence. The 'B' tip, which is recommended for the investigation of smooth surface lesions, was used for the analyses. Samples were analysed in numerical order of their assigned random numbers allowing the blind analysis of each sample with respect to its demineralisation solution and demineralisation time. Each sample was removed from its individual container and gently dried in a stream of compressed air for 3 seconds. Clinically sound enamel on the cusp tip of each sample was identified and used as the point on which to carry out the adaptation or 'zeroing' of the DIAGNOdent device. The probe tip was then passed over the artificial carious lesion on the buccal surface of the specimen. The probe position and angulation was varied and the peak reading was recorded for each specimen. In the majority of specimens the test site was clearly visible indicating the area for analysis but in others where little or no area of demineralisation was obvious, the middle third was scanned with the DIAGNOdent probe as it was known to be the site on the tooth surface which had been exposed to the demineralising solution. After recording the peak DIAGNOdent

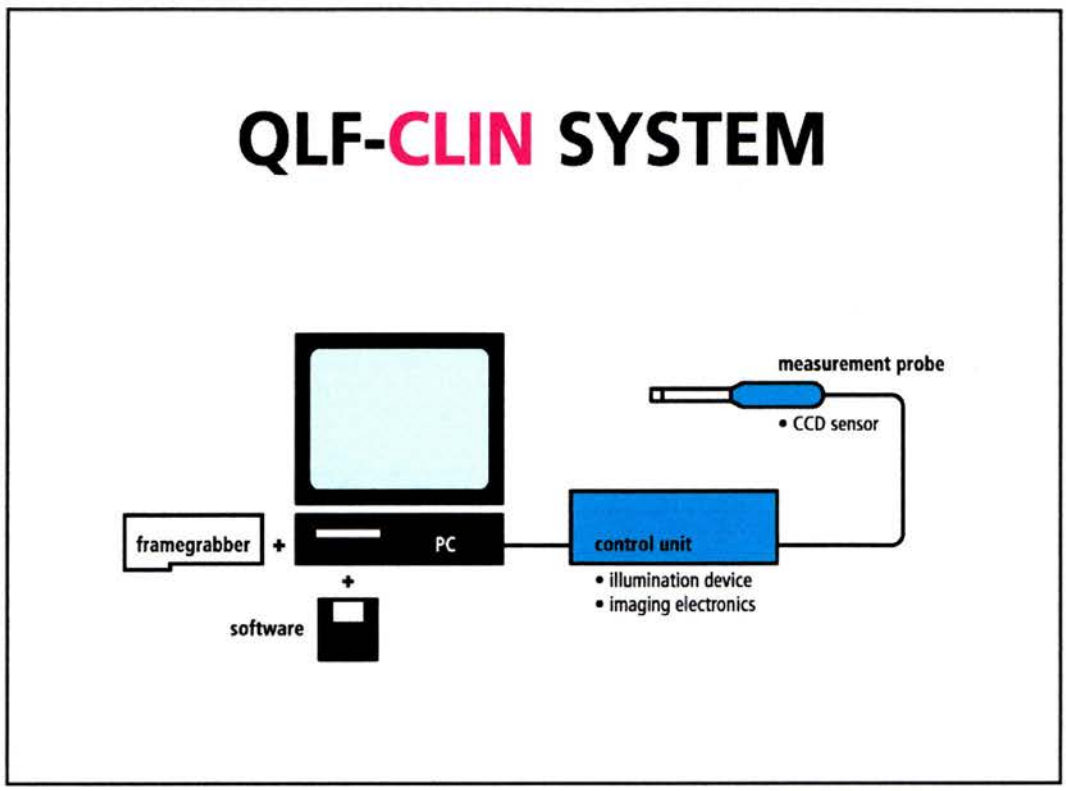
value, the instrument was re-zeroed on sound enamel and the analysis was repeated. The peak value reached on each occasion was recorded. 4 different examiners carried out an analysis of the 220 samples according to this protocol giving a total of 8 separate values for each specimen. All values were recorded and entered into a computer spreadsheet (Microsoft Excel 5.0) for analysis.

2.2.7.2 QLF Analysis

All samples were further analysed by the quantitative light fluorescence technique described by de Josselin de Jong et al., (1995). The QLF \ clin system used for lesion analysis is illustrated in figure 2.11 and represented diagrammatically in figure 2.12

Figure 2.11 QLF \ clin System for Lesion Analysis

Figure 2.12 Diagrammatic Representation of Operation of QLF \ clin System



The examination using the QLF system was carried out in a darkened room. Each specimen was removed from its sample bottle and gently dried in a stream of compressed air for 3 seconds. The specimen was placed on an adjustable platform using a small piece of 'Blu-tack' to stabilise its position. The CCD camera (Panasonic GP-KS152, Panasonic Communications Systems Co., Secaucus, NJ, USA) of the QLF system was held in a clamp stand and positioned over the tooth specimen (figure 2.13).

Fig. 2.13 Specimen Positioned for QLF Analysis

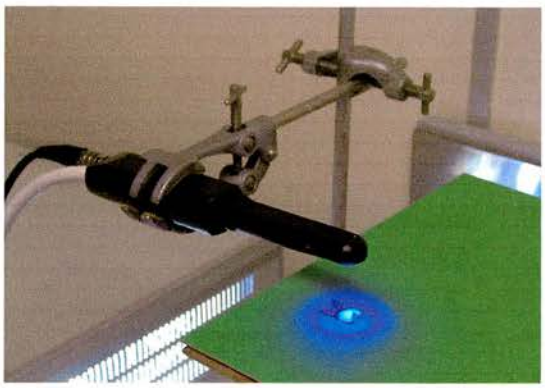
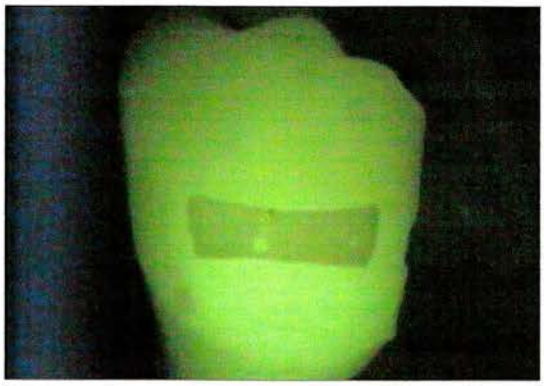


Fig. 2.14 Image of Demineralised Lesion Grabbed by QLF System for Analysis



The resultant image (figure 2.14) was displayed on the screen of a personal computer connected to the system and the position of the tooth on its adjustable platform was adjusted to optimise the clarity and focus of this image. The position of the specimen was also adjusted to ensure the central placement of the demineralised lesion within the illumination from the light source of the QLF system. Once the clearest and most evenly illuminated image was obtained, this was frozen by a frame-grabbing device attached to the system and stored on the hard disc of the computer for subsequent analysis. The specimen was returned to its storage sample bottle. Images of all 220 specimens were recorded in this way.

The resultant images of each lesion were subsequently analysed. Computer software, designed for image analysis, (Quantitative Light-induced Fluorescence v.1.97i, Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to quantify the change in fluorescence and lesion size for the recorded images. With the frozen image of the lesion displayed on the computer screen, the computer mouse was used

to outline the lesion by dragging a cursor to create an envelope around the lesion. The envelope was then adjusted so that it closely followed the borders of the lesion and was placed just into the surrounding sound enamel to define a reference flourescence value for sound tissue against which the fluorescence of the lesion was compared.

The software then enabled the computer to reconstruct a sound tooth on the image of the lesion by comparing the fluorescence of individual points with the fluorescence of the adjacent sound tissue as defined by the placement of the surrounding envelope. (figures 2.15-2.18)

Fig. 2.15 Carious Lesion

Fig. 2.16 QLF Image of Lesion

Fig. 2.17 Envelope Placed Around Lesion

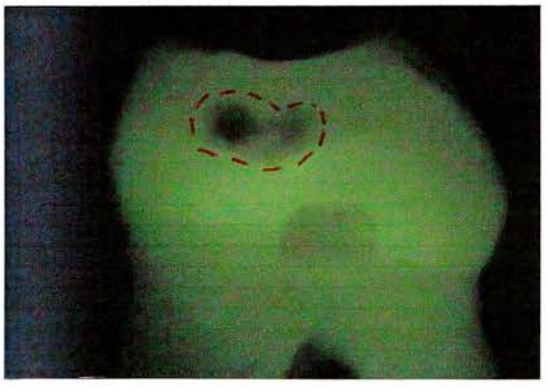
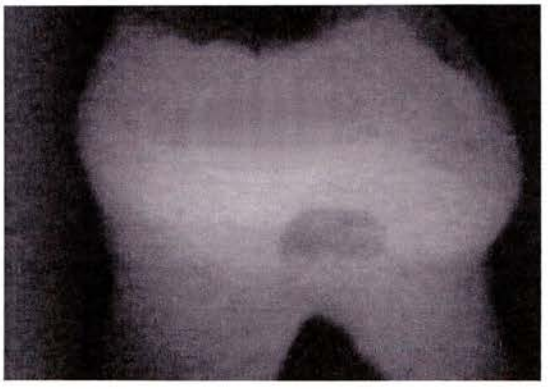


Fig. 2.18 Reconstructed Image of Lesion in Grey Scales



The decrease in fluorescence was determined by calculating the percentage difference between the actual and reconstructed fluorescence of each point. Three parameters were quantified as described by de Josselin de Jong et al., (1995).

1. ΔL_{max} (%) is the largest decrease in fluorescence recorded for the lesion.
2. ΔL_{mean} (%) is the mean decrease in fluorescence of all points within the lesion area.
3. Lesion Area (mm^2) is the area of tooth surface with at least 10% decrease in fluorescence.

ΔL_{max} and ΔL_{mean} values were expressed as percentages of the reconstructed values. The lesion area was calculated by measuring the surface occupied by points with a difference larger than 10% of the reconstructed values for sound enamel. Conversion of this area into square millimetres was carried out automatically by the computer software. The software automatically reported lesion area, ΔL_{max} and ΔL_{mean} for each lesion.

2.3 Results

The distribution of the data was examined and the Shapiro-Wilk statistic was calculated to determine whether or not the data were normally distributed. Results indicated that the data were extremely skewed and therefore non-parametric statistical tests were used.

2.3.1 DIAGNOdent Analysis

The mean and median DIAGNOdent values for each demineralisation time period were calculated for each of the four examiners. Results are shown in tables 2.1-2.4.

Table 2.1. Mean and Median DIAGNOdent Values for Each Demineralisation Time (Examiner 1)

Demineralisation Time (Hours)	Mean DIAGNOdent Value (S.E.M.)		Median DIAGNOdent Value	
	Reading I	Reading II	Reading I	Reading II
0	0.45 (0.14)	0.35 (0.17)	0	0
8	0.73 (0.19)	0.85 (0.17)	0	0.5
24	0.93 (0.20)	0.95 (0.20)	1	1
48	0.85 (0.26)	0.80 (0.28)	1	0
72	0.63 (0.14)	0.95 (0.22)	0	0.5
96	0.95 (0.18)	0.83 (0.16)	1	1.0

Table 2.2. Mean and Median DIAGNOdent Values for Each Demineralisation Time (Examiner 2)

Demineralisation Time (Hours)	Mean DIAGNOdent Value (S.E.M.)		Median DIAGNOdent Value	
	Reading I	Reading II	Reading I	Reading II
0	0.80 (0.19)	0.55 (0.18)	1	0
8	0.90 (0.19)	0.95 (0.19)	1	1
24	1.15 (0.19)	1.43 (0.20)	1	1
48	1.70 (0.42)	1.63 (0.46)	1	1
72	0.95 (0.15)	1.20 (0.21)	1	1
96	1.58 (0.41)	1.45 (0.32)	1	1

Table 2.3. Mean and Median DIAGNOdent Values for Each Demineralisation Time (Examiner 3)

Demineralisation Time (Hours)	Mean DIAGNOdent Value (S.E.M.)		Median DIAGNOdent Value	
	Reading I	Reading II	Reading I	Reading II
0	0.05 (0.05)	0.35 (0.15)	0	0
8	0.75 (0.23)	0.90 (0.18)	0	1
24	0.85 (0.19)	0.70 (0.20)	1	0
48	2.53 (1.34)	2.80 (1.37)	1	1
72	1.45 (0.36)	1.63 (0.43)	1	1
96	2.23 (1.27)	2.25 (1.29)	1	1

Table 2.4. Mean and Median DIAGNOdent Values for Each Demineralisation Time (Examiner 4)

Demineralisation Time (Hours)	Mean DIAGNOdent Value (S.E.M.)		Median DIAGNOdent Value	
	Reading I	Reading II	Reading I	Reading II
0	0.40 (0.20)	0.25 (0.14)	0	0
8	0.57 (0.21)	0.68 (0.18)	0	1
24	0.35 (0.22)	0.68 (0.22)	0	0
48	1.78 (0.63)	1.85 (0.68)	1	1
72	1.27 (0.22)	0.98 (0.24)	1	1
96	1.18 (0.20)	1.18 (0.25)	1	1

2.3.1.1 Intra-Examiner Agreement for DIAGNOdent Analysis

The correlation between the first and second DIAGNOdent analysis carried out for each specimen was calculated for each of the four examiners. Results are shown in table 2.5.

Table 2.5. Intra-Examiner Correlation for DIAGNOdent Analysis

Examiner	Spearman's Correlation Coefficient (Reading 1 vs Reading 2)	p value
1	0.49	0.000
2	0.43	0.000
3	0.63	0.000
4	0.63	0.000

2.3.1.2 Inter-Examiner Agreement for DIAGNOdent Analysis

The correlation of DIAGNOdent analysis of specimens between different examiners was calculated and significance determined. In addition, the Wilcoxon Signed Ranks Test was used to determine the existence of significant differences between the results of the various examiner pairs. Results are shown in table 2.6.

Table 2.6. Inter-Examiner Correlation for DIAGNOdent Analysis

Examiners	Spearman's Correlation Coefficient (p value)	Wilcoxon Signed Ranks Test p value
1 vs 2	0.56 (0.000)	0.000
1 vs 3	0.28 (0.000)	0.455
1 vs 4	0.28 (0.000)	0.166
2 vs 3	0.38 (0.000)	0.085
2 vs 4	0.35 (0.000)	0.074
3 vs 4	0.42 (0.000)	0.670

2.3.1.3 Correlation of DIAGNOdent Analysis with Demineralisation Time

The correlation between DIAGNOdent value and demineralisation time was calculated for all lesions irrespective of lesion creation protocol (surface abrasion and demineralisation solution). As there appeared to be interactions between the type of demineralisation solution and the use of surface abrasion, the influence of the different lesion creation protocols was studied and correlations for individual groups on the basis of surface abrasion and demineralisation solution type were also determined. The Kruskal Wallis test was used to determine the existence of any significant differences between groups on the basis of lesion creation protocol. Results are shown in table 2.7.

Table 2.7. Correlation of DIAGNOdent Value with Demineralisation Time for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s (p value)	KW p value
All	220	0.24 (0.000)	0.003
Abraded / 'Carbopol'	60	0.37 (0.003)	0.097
Abraded / 'Glasgow'	60	0.25 (0.593)	0.015
Unabraded / 'Carbopol'	60	0.15 (0.244)	0.042
Unabraded / 'Glasgow'	60	0.47 (0.000)	0.006

2.3.2 QLF Analysis

The mean and median QLF parameters (mean loss of fluorescence, maximum loss of fluorescence and lesion area) for all specimens, irrespective of demineralising solution or enamel surface preparation according to demineralisation time were calculated for each examiner. Results are shown in tables 2.8-2.15.

Table 2.8. Mean QLF Parameters for all Lesions (Examiner 1)

Demin Time (Hours)	ΔL_{mean} (%) (S.E.M.)	ΔL_{max} (%) (S.E.M.)	Lesion Area (mm^2) (S.E.M.)
0	-10.90 (0.18)	-15.80 (0.99)	0.05 (0.02)
8	-11.76 (0.34)	-20.33 (2.03)	0.68 (0.26)
24	-12.71 (0.33)	-26.28 (1.66)	1.25 (0.22)
48	-15.44 (0.59)	-31.43 (1.45)	2.80 (0.37)
72	-16.11 (0.56)	-33.70 (1.57)	2.69 (0.30)
96	-18.57 (0.82)	-39.48 (1.68)	3.98 (0.34)

Table 2.9. Median QLF Parameters for all Lesions (Examiner 1)

Demin Time (Hours)	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
0	-10.70	-15.00	0.00
8	-11.00	-17.00	0.10
24	-12.05	-24.50	0.65
48	-14.40	-31.50	2.40
72	-15.45	-34.00	2.90
96	-17.55	-40.00	4.40

Table 2.10. Mean QLF Parameters for all Lesions (Examiner 2)

Demin Time (Hours)	ΔL_{mean} (%) (S.E.M.)	ΔL_{max} (%) (S.E.M.)	Lesion Area (mm^2) (S.E.M.)
0	-11.01 (0.29)	-15.50 (1.02)	0.11 (0.04)
8	-11.98 (0.29)	-21.65 (1.48)	0.72 (0.19)
24	-12.98 (0.35)	-27.48 (1.85)	1.33 (0.22)
48	-15.63 (0.54)	-31.80 (1.33)	2.87 (0.30)
72	-16.49 (0.64)	-36.13 (1.62)	2.78 (0.35)
96	-19.18 (0.95)	-39.53 (1.73)	3.91 (0.37)

Table 2.11. Median QLF Parameters for all Lesions (Examiner 2)

Demin Time (Hours)	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
0	-10.90	-15.00	0.00
8	-11.50	-20.00	0.10
24	-12.25	-26.00	1.10
48	-14.65	-31.00	2.95
72	-15.70	-35.00	2.65
96	-18.70	-39.00	4.25

Table 2.12. Mean QLF Parameters for all Lesions (Examiner 3)

Demin Time (Hours)	ΔL_{mean} (%) (S.E.M.)	ΔL_{max} (%) (S.E.M.)	Lesion Area (mm^2) (S.E.M.)
0	-13.36 (1.84)	-21.30 (3.99)	0.46 (0.25)
8	-11.79 (0.32)	-20.80 (1.62)	0.45 (0.20)
24	-13.58 (0.94)	-28.53 (2.09)	1.03 (0.15)
48	-15.29 (0.57)	-31.40 (1.40)	2.34 (0.30)
72	-16.10 (0.61)	-35.23 (1.63)	2.46 (0.30)
96	-18.14 (0.85)	-36.70 (1.62)	3.50 (0.38)

Table 2.13. Median QLF Parameters for all Lesions (Examiner 3)

Demin Time (Hours)	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
0	-10.70	-14.50	0.00
8	-11.10	-18.50	0.15
24	-12.45	-27.50	0.95
48	-14.10	-30.50	1.50
72	-14.85	-35.50	2.25
96	-17.90	-37.50	3.80

Table 2.14. Mean QLF Parameters for all Lesions (Examiner 4)

Demin Time (Hours)	ΔL_{mean} (%) (S.E.M.)	ΔL_{max} (%) (S.E.M.)	Lesion Area (mm^2) (S.E.M.)
0	-14.20 (1.38)	-27.40 (3.71)	0.50 (0.20)
8	-14.83 (1.16)	-28.08 (2.75)	1.02 (0.28)
24	-14.98 (0.70)	-34.13 (2.25)	1.17 (0.17)
48	-17.59 (0.99)	-38.10 (2.59)	1.94 (0.24)
72	-16.42 (0.55)	-39.83 (1.77)	1.71 (0.21)
96	-19.27 (1.19)	-42.03 (2.32)	2.31 (0.26)

Table 2.15. Median QLF Parameters for all Lesions (Examiner 4)

Demin Time (Hours)	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
0	-12.00	-23.00	0.10
8	-11.80	-21.00	0.10
24	-13.10	-28.50	1.05
48	-15.40	-36.50	1.95
72	-15.90	-41.00	1.55
96	-16.05	-41.50	2.00

2.3.2.1 Inter-Examiner Agreement for QLF Analysis

The correlation of QLF analysis of specimens between different examiners was calculated. In addition, the Wilcoxon Signed Ranks test was used to determine the existence of significant differences between the results of the individual examiners Results are shown in tables 2.16-2.18

Table 2.16. Inter-Examiner Correlation for ΔL_{mean} (%)

Examiners	Spearman's Correlation Coefficient (p value)	Wilcoxon Signed Ranks Test p value
1 vs 2	0.81 (0.000)	0.173
1 vs 3	0.73 (0.000)	0.165
1 vs 4	0.35 (0.000)	0.001
2 vs 3	0.71 (0.000)	0.066
2 vs 4	0.32 (0.000)	0.018
3 vs 4	0.37 (0.000)	0.020

Table 2.17. Inter-Examiner Correlation for ΔL_{max} (%)

Examiners	Spearman's Correlation Coefficient (p value)	Wilcoxon Signed Ranks Test p value
1 vs 2	0.73 (0.000)	0.108
1 vs 3	0.67 (0.000)	0.259
1 vs 4	0.33 (0.000)	0.000
2 vs 3	0.68 (0.000)	0.258
2 vs 4	0.28 (0.000)	0.000
3 vs 4	0.32 (0.000)	0.000

Table 2.18. Inter-Examiner Correlation for Lesion Area (mm^2)

Examiners	Spearman's Correlation Coefficient (p value)	Wilcoxon Signed Ranks Test p value
1 vs 2	0.79 (0.000)	0.596
1 vs 3	0.76 (0.000)	0.127
1 vs 4	0.42 (0.000)	0.000
2 vs 3	0.73 (0.000)	0.005
2 vs 4	0.35 (0.000)	0.000
3 vs 4	0.38 (0.000)	0.020

2.3.2.2 Correlation of QLF Analysis with Demineralisation Time

The correlation between QLF parameter values and demineralisation time was calculated for all lesions irrespective of lesion creation protocol (surface abrasion and demineralisation solution). As there appeared to be interactions between the type of demineralisation solution and the use of surface abrasion, the influence of the different lesion creation protocols was studied and correlations for individual groups on the basis of surface abrasion and demineralisation solution type were also determined. The Kruskal Wallis test was used to determine the existence of any significant differences between groups on the basis of lesion creation protocol. Results are shown in tables 2.19-2.21.

Table 2.19. Correlation of ΔL_{mean} (%) with Demineralisation Time for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s (p value)	KW p value
All	220	-0.60 (0.000)	0.000
Abraded / 'Carbopol'	60	-0.66 (0.000)	0.000
Abraded / 'Glasgow'	60	-0.71 (0.000)	0.000
Unabraded / 'Carbopol'	60	-0.61 (0.000)	0.000
Unabraded / 'Glasgow'	60	-0.61 (0.000)	0.000

Table 2.20. Correlation of ΔL_{max} (%) with Demineralisation Time for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s (p value)	KW p value
All	220	-0.55 (0.000)	0.000
Abraded / 'Carbopol'	60	-0.63 (0.000)	0.000
Abraded / 'Glasgow'	60	-0.68 (0.000)	0.000
Unabraded / 'Carbopol'	60	-0.56 (0.000)	0.001
Unabraded / 'Glasgow'	60	-0.58 (0.000)	0.000

Table 2.21. Correlation of Lesion Area (mm^2) with Demineralisation Time for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s (p value)	KW p value
All	220	0.62 (0.000)	0.000
Abraded / 'Carbopol'	60	0.70 (0.000)	0.000
Abraded / 'Glasgow'	60	0.73 (0.000)	0.000
Unabraded / 'Carbopol'	60	0.61 (0.000)	0.000
Unabraded / 'Glasgow'	60	0.60 (0.000)	0.000

2.3.3 Correlation of DIAGNOdent Analysis with QLF Analysis

Correlations between DIAGNOdent analysis and the three measured QLF parameters (ΔL_{mean} (%), ΔL_{max} (%), and Lesion Area (mm^2)) for all lesions and individual groups based on lesion creation protocol were calculated. Results are shown in table 2.22-2.24.

Table 2.22. Correlation of DIAGNOdent Value with ΔL_{mean} (%) for Different Lesion Creation Protocols

Lesion Group	Number of Lesions	r^s	p value
All	220	-0.22	0.001
Abraded / 'Carbopol'	60	-0.10	0.466
Abraded / 'Glasgow'	60	-0.12	0.357
Unabraded / 'Carbopol'	60	-0.42	0.001
Unabraded / 'Glasgow'	60	-0.56	0.000

Table 2.23. Correlation of DIAGNOdent Value with ΔL_{max} (%) for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s	p value
All	220	-0.21	0.002
Abraded / 'Carbopol'	60	-0.12	0.379
Abraded / 'Glasgow'	60	-0.11	0.391
Unabraded / 'Carbopol'	60	-0.45	0.000
Unabraded / 'Glasgow'	60	-0.56	0.000

Table 2.24. Correlation of DIAGNOdent Value with Lesion Area (mm^2) for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s	p value
All	220	0.19	0.006
Abraded / 'Carbopol'	60	0.18	0.174
Abraded / 'Glasgow'	60	0.03	0.797
Unabraded / 'Carbopol'	60	0.33	0.011
Unabraded / 'Glasgow'	60	0.58	0.000

2.4 Discussion

2.4.1 Enamel Source

Most experimental work in cariology makes use of either bovine enamel obtained from abattoirs or human enamel from extracted teeth. Bovine enamel has been available in large quantities and is of homogeneous structure making it an attractive medium for study. However, Featherstone and Mellberg (1981) compared human and bovine enamel demonstrating differences in their rates of demineralisation and for this reason, the use of non-human material for study may raise questions over the validity of applying findings to human subjects. Furthermore, in recent years, some concern has been raised over the theoretical risk, when using bovine teeth for experimental work, of transmission of agents associated with bovine spongiform encephalopathy.

Although resources in respect of extracted human teeth are limited, fortunately a reasonably abundant supply was available locally (water fluoridation <0.03ppm) and it was therefore decided to use enamel derived from extracted human teeth for the experimental work to be undertaken. Nevertheless, the quality of the available teeth for study was variable and considerable sorting of the specimens was necessary to derive suitable specimens. Storage of extracted teeth, until required for use, was effected in 0.12% thymol. This offered a bacteriostatic environment without the associated problems of tenacious adherence of proteinacious debris sometimes

associated with formalin fixation (Eggertsson et al., 1999). This may have the potential to interfere with artificial lesion creation.

2.4.2 Creation of Artificial Lesions

The range of methods for production of artificial lesions and some of the differences between techniques have been discussed in section 1.3. The laboratory where this experimental work was undertaken had experience of using the acetic acid based 'Glasgow Solution' system of Damato et al., (1988) and also the 'Carbopol' system of White (1987). Due to local expertise in preparation of these systems along with documented evidence of their success, it was decided to prepare artificial lesions using both these methods. The observed effect of varying the demineralising solution is discussed, along with the effect of enamel surface abrasion in section 2.4.3.4

The nail varnish chosen for the varnishing of the specimens was Max Factor Firebrand since not only is this product acid resistant but communication with the cosmetics company has suggested that no fluoride is included in its manufacture. The bright red colour provided a suitable contrast to the tooth surface. An attempt was made to standardise both size and placement of the bare enamel window, placing it in the middle third of the buccal wall with respect to the long axis of the tooth. During lesion analysis, the majority of artificial lesions were visible with the naked eye but in those specimens where the lesion was less distinct, this consistency of positioning aided the location of the area for analysis.

The 5 different demineralisation time periods chosen were 8, 24, 48, 72 and 96 hours. Christoffersen and Arends (1982) demonstrated a linear relationship between lesion depth and time$^{1/3}$ and this was confirmed by White (1987). Therefore, the demineralising time periods were chosen to give a spread of expected lesion depths. Hall et al., (1997) described demineralisation periods with a smaller spread but the ability of the DIAGNOdent to discriminate between the smaller time periods has not been established.

2.4.3 DIAGNOdent Analysis

Given the relative ease and speed of lesion analysis with the DIAGNOdent in comparison to other fluorescence techniques (e.g. QLF-clin) it was considered appropriate to carry out consecutive DIAGNOdent analyses of each specimen in order to gain a more accurate assessment of the lesion status. Double analysis by each individual in conjunction with analysis by 4 separate examiners allowed a comprehensive assessment with each lesion undergoing a total of 8 analyses.

Thorough drying of all specimens was ensured prior to DIAGNOdent analysis. The effect of lesion moisture content on light scattering and subsequent optical analysis has been described by Angmar-Månsson and ten Bosch (1987). In addition, Shi et al., (2000a) specifically studied the effect of wet and dry conditions on DIAGNOdent analysis and reported significant differences dependant on the condition of the tooth surface during analysis. The manufacturers of the DIAGNOdent also recommend a

clean, dry tooth surface for analysis and efforts were made to ensure consistency in this respect.

2.4.3.1 Intra-Examiner Agreement

The 2 separate analyses of each specimen carried out by each examiner were subjectively similar and consideration was given to the handling of these paired results. Calculation of Spearman's correlation coefficient for the two analyses (table 2.5) indicated highly significant correlations ($p<0.001$) between the two readings for all examiners and it was therefore considered appropriate to use only the first data set for each examiner in subsequent analyses. This allowed considerably more straightforward data analysis.

Other authors have studied the repeatability of the DIAGNOdent system. Lussi et al., (1998b) demonstrated a high intra-examiner reproducibility with DIAGNOdent analysis. Using a sample consisting of 83 extracted molars with natural occlusal caries, they reported Spearman's correlation coefficients of between 0.83 and 0.99 for 11 different examiners.

Shi et al., (2000a) also reported reproducibility of DIAGNOdent analysis. This paper reported higher correlation between sequential analyses using DIAGNOdent than was found in this study. However, the sample was different in that natural occlusal caries in extracted teeth was studied and the sample size was considerably smaller.

Shi et al., (2000a) also quoted *Pearson's* correlation coefficient and this use of a parametric test suggests a more normal distribution of data was evident in their results.

2.4.3.2 Inter-Examiner Agreement

Results were subjectively similar for each of the 4 individual examiners and inter-examiner agreement was tested by calculating Spearman's correlation coefficient for all possible examiner pairs (table 2.6). Results indicated highly significant correlations ($p<0.001$) between each examiner pair. In addition, the Wilcoxon Signed Ranks Test results (table 2.6) also indicated (with the exception of examiners 1 and 2) that there were no significant differences between the results of paired examiners. It was therefore considered reasonable to use the results of only one examiner in subsequent data analyses. The results of the author (examiner 3) were chosen.

The inter-examiner reproducibility of DIAGNOdent analysis was also investigated by Verdonschot et al., (1999). They reported a correlation coefficient between examiners of 0.79. This study was carried out *in vivo* and involved a sample size of only 41 teeth. However, according to the method described, only 25% of measurements were repeated suggesting a vary small sample size for assessing repeatability.

Lussi et al., (1999a) also studied the inter-examiner repeatability of DIAGNOdent analysis. In this study, different dentists examined 83 extracted molars with non-cavitated occlusal caries. The inter-examiner Spearman's correlation coefficient was reported to be 0.84. This was higher than that found here, but the sample size was smaller and the significance of the result was not stated.

2.4.3.3 Correlation of DIAGNOdent Value with Demineralisation Time

The DIAGNOdent value obtained from analysis of a carious lesion is claimed to reflect the degree of mineralisation of that specimen. The gold standard of mineral loss in this experiment was taken to be evidence of exposure to a known demineralising solution. It was therefore expected to observe a correlation between the length of time to which an enamel specimen was exposed to the demineralising solution (a reflection of the degree of mineral loss) and the DIAGNOdent value obtained during subsequent analysis of that specimen.

The DIAGNOdent values for all lesions irrespective of the demineralising protocols of the study were examined in relation to the demineralisation time period. Subjective analysis of calculated mean and median values for DIAGNOdent analysis indicated a slight trend of increasing DIAGNOdent value with increasing demineralisation time (tables 2.1-2.4). However, this simple preliminary analysis of means and medians was not suggestive of a strong correlation.

Nevertheless, statistical analysis of DIAGNOdent values in relation to demineralisation time for all lesions did indicate a significant correlation between these parameters (table 2.7). For all 220 specimens, regardless of lesion creation protocol, the Spearman's correlation co-efficient was calculated to be 0.24. This is an extremely low level of correlation which indicates that the DIAGNOdent value did not appear to closely reflect the degree of demineralisation. However, the large sample size for this group resulted in a high significance for this correlation ($p<0.001$).

The Kruskal Wallis test was also used to assess the ability of the DIAGNOdent to discriminate between the different demineralisation periods and results using this test also indicated significant differences between the DIAGNOdent values for each demineralisation period ($p<0.01$). Thus the validity of DIAGNOdent value as a measure of the degree of demineralisation of a given lesion was demonstrated with a low (but significant) correlation with exposure time to demineralising solution. Correlation of DIAGNOdent value with mineral loss has also been studied elsewhere.

Correlation of DIAGNOdent value with mineral loss as measured by TMR was demonstrated by Ando et al., (1999). This study also used artificially created carious lesions in extracted teeth and TMR analysis was used as the gold standard of demineralisation. The correlation of DIAGNOdent value to mineral loss was found to be 0.58 and this was inferior to that of the other fluorescence techniques (QLF) also

tested in this study. However, Ando et al., (1999) demineralised their specimens for up to 120 hours which was longer than the maximum of 96 hours used in the present study and the possibility exists that the additional demineralisation time may have had some effect.

Tranæus et al., (2000) also determined the correlation of DIAGNOdent value with mineral loss and they too found this to be 0.58. In this study, 71 non-cavitated smooth surface lesions in extracted premolars were studied using both DIAGNOdent and QLF. In addition both histological and TMR analyses were carried out on sections from each lesion to determine lesion depth and mineral loss which served as the gold standard. The correlation between DIAGNOdent value and mineral loss was identical to that reported by Ando et al., (1999) despite the use of naturally occurring lesions in comparison to the artificially created lesions used in the latter study. Discussion has already been made in section 1.3 of the validity of comparing natural and artificial carious lesions. Nevertheless, Tranæus et al., (2000) concluded that the other fluorescence technique under study (QLF) was superior to DIAGNOdent analysis in terms of correlation with mineral loss and lesion depth.

2.4.3.4 Influence of Lesion Creation Protocol

Damato et al., (1988) has suggested that the method of lesion preparation has an influence on the subsequent behaviour of lesions when exposed to demineralising and remineralising protocols. A further variable studied in terms of lesion preparation

was the effect of enamel surface abrasion. High concentrations of fluoride in the surface layers of enamel may create problems in artificial lesion creation (White 1987) and surface abrasion has the potential to remove this fluoride rich layer. Previous studies have employed surface treatment with pumice (Silverstone 1982); brushing with warm detergent solution (White 1987) and micro-abrasion by sandblasting with 50 micron aluminium oxide powder (Eggertson et al., 1999). In this study surface micro-abrasion using diamond burs was undertaken. Depth gauged veneer preparation burs were chosen in order to standardise the amount of enamel surface removed. These burs, by virtue of their banded design, are unable to cut beyond 300μm. The use of these burs in a high speed turbine with water coolant was expected to remove all organic debris along with the outermost 300μm of fluoride rich enamel leaving an exposed surface more receptive to lesion creation by the acid of the demineralising solution.

The use of the two distinct demineralising protocols with variation in surface preparation and demineralising solution offered the potential for comparison in respect of the fluorescence analysis. Analysis of the relationship between DIAGNOdent value and demineralisation time for all 220 lesions suggested the existence of interactions between the type of demineralisation solution and the surface abrasion used in lesion creation. Therefore, the influence of each separate lesion creation protocol was studied. Table 2.7 also shows the correlation between DIAGNOdent value and demineralisation time for each individual lesion creation protocol.

The influence of surface abrasion was inconsistent. For 'Carbopol' created lesions, abraded specimens demonstrated a higher correlation (0.37, $p<0.01$) than unabraded specimens (0.15, $p=0.244$). Conversely for 'Glasgow' created lesions, abraded specimens demonstrated a poorer correlation. Differences between groups undergoing different surface abrasion may be accounted for by considering the more homogeneous nature of the subsurface enamel. The surface enamel is exposed to a variety of extrinsic factors *in situ* and removal of the outer 300μm of enamel from each specimen would be expected to reduced the influence of this heterogeneous sample matter resulting in more predictable lesion behaviour and a stronger correlation with demineralisation time.

The influence of demineralising solution was also inconsistent. For abraded lesions, the use of 'Carbopol' solution for demineralisation resulted in a significantly better correlation with demineralisation time than the use of 'Glasgow' solution. However, exactly the converse was found for unabraded lesions.

The Kruskal Wallis test was also used to analyse the ability of the DIAGNOdent to distinguish significant differences between the various demineralisation times for each of the 4 lesion creation protocols (table 2.7). Using this test, varying significant differences between time periods were evident for all protocols except 'Abraded / Carbopol'. Thus the influence of surface abrasion and demineralising solution was uncertain and no clear conclusions were evident.

There has been no previous direct comparison of different demineralisation solutions in respect of subsequent fluorescence analysis. However, other authors have studied the influence of demineralisation solution on other aspects of lesion characteristics. Manson-Hing et al., (1972) compared lesions created by a lactic acid acidified gel system (c.f. 'Carbopol Solution') and an acetic acid based buffered system (c.f. 'Glasgow Solution'). The gel based solution resulted in a much better degree of surface preservation than the liquid buffered solution as demonstrated by microradiography. It seems reasonable to postulate that such a difference in structure of the artificial lesion may be responsible for an altered interpretation by techniques involving a degree of light scattering (e.g. DIAGNOdent).

The actual degree of demineralisation effected in the given exposure time to each solution may also have had a potential influence on the subsequent DIAGNOdent analysis. Damato et al., (1988) studied the rates of mineral loss in two different methods of artificial lesion creation and concluded that significant differences existed between an acidified gelatin system and a buffered solution system. They also suggested that high fluoride levels in gel systems can result in arresting of the artificial lesion whilst still in the gelatin medium resulting in a lesser degree of demineralisation in the same exposure time. This must be considered to be a theoretical possibility for influencing the DIAGNOdent analysis of these lesions.

2.4.4 QLF Analysis

A number of factors may be expected to potentially influence the QLF analysis of the lesions under study. Extraneous light sources may theoretically have an effect on the wavelength and intensity of light detected by the CCD camera incorporated into the system. The analysis of specimens using the QLF \ clin system was therefore undertaken in a darkened room to reduce the possible effect of extraneous light sources. Furthermore, the degree of hydration of the lesions may be expected to alter the light transmitting properties of the demineralised enamel. Light scattering is increased in drier lesions due to the relatively larger difference in refractive index of air to crystals than water to crystals (Angmar-Månsson and ten Bosch 1987). Smaller lesions may be expected to dry out more quickly than larger lesions (van der Veen et al., 1997) and therefore this may have had the effect of reducing observed differences between lesions of differing size. However, efforts were made during production of the bare enamel windows in lesion creation to ensure consistency of lesion size and in addition all specimens were thoroughly dried with compressed air before analysis. Nevertheless it was observed that when placed under the intense light source of the QLF \ clin system, lesion images on screen appeared to darken slightly after a few seconds as residual moisture evaporated. This was not considered to be a problem since a stable situation was reached by the time each specimen's position was adjusted to maximise image positioning and clarity.

2.4.4.1 Inter-Examiner Agreement

Results of QLF analysis were subjectively similar for each of the 4 individual examiners and inter-examiner agreement was tested by calculating Spearman's correlation coefficient for all possible examiner pairs for each QLF parameter (tables 2.16-2.18). Results indicated highly significant correlations between each examiner pair and it was therefore considered reasonable to use the results of only one examiner in subsequent data analyses. The results of the author (examiner 3) were chosen.

2.4.4.2 Correlation of QLF Parameters and Demineralisation Time

The QLF parameter values for all lesions irrespective of the different lesion creation protocols were examined in relation to the demineralisation time period. Subjective analysis of calculated mean and median values for ΔL_{mean} (%) , ΔL_{max} (%) and Lesion Area (mm^2) indicated a trend of increasing parameter value with increasing demineralisation time (tables 2.8-2.15).

Statistical analysis of QLF parameter values in relation to demineralisation time for all lesions indicated a significant correlation between these parameters and demineralisation time (tables 2.19-2.21). For all 220 specimens, regardless of lesion creation protocol, the Spearman's correlation co-efficient for ΔL_{mean} (%) was -0.60 ($p<0.001$), for ΔL_{max} (%) it was -0.55 ($p<0.001$) and for Lesion Area (mm^2) it was

0.62 ($p<0.001$). The Kruskal Wallis test was also used to determine the presence of significant differences between the groups based on demineralisation time. Kruskal Wallis test results were highly significant ($p<0.001$) for the ability of all 3 measured QLF parameters to reflect the varying demineralisation time (tables 2.19-2.21). On this basis it was concluded that QLF\clin was a valid means of assessing the degree of demineralisation of artificially created lesions.

Lagerweij et al., (1999) also studied the correlation between the QLF\clin system and mineral loss. These workers also used lesions created artificially with 'Carbopol' demineralising solution and a surface abrasion protocol was also employed. Lagerweij et al., (1999) calculated the Pearson's correlation coefficient to be 0.63 which is similar to the 0.60 obtained in the work described here. Similar correlations of 0.65 and 0.76 were reported in work described by ten Cate et al., (1996) and Al-Khateeb et al., (1997b) respectively.

2.4.4.3 Influence of Lesion Creation Protocol

The influence of lesion creation protocol on DIAGNOdent analysis was discussed in 2.4.3.4. The effect of lesion creation protocol on QLF analysis was also investigated and the correlation with demineralisation time was calculated for individual groups based on demineralising solution and use of surface abrasion (tables 2.19-2.21). All groups demonstrated a significant correlation with demineralisation time ($p<0.001$) and the different lesion creation protocols did not appear to have a significant effect.

Spearman's correlation coefficient varied between 0.60 and 0.71 for ΔL_{mean} (%) ; 0.55 and 0.68 for ΔL_{max} (%) and 0.60 and 0.73 for Lesion Area (mm^2). This is in contrast to the DIAGNOdent analysis of the same lesions where correlation varied more with lesion creation protocol (range 0.15 to 0.47).

2.4.5 Correlation between DIAGNOdent and QLF Analyses

Significant correlations between DIAGNOdent and QLF analysis were found for all measured QLF parameters (tables 2.22-2.24). Although statistically significant, correlation values were not high and the agreement between the two techniques did not appear to be strong. In addition, differences in the degree of correlation were noted when individual groups were analysed on the basis of lesion creation protocol. For all three QLF parameters, significant correlations were only observed in the 'unabraded' groups. Varying the demineralisation solution did not appear to have a significant effect on the observed correlation between the methods of lesion analysis. As discussed previously (2.4.3.4), surface abrasion would be expected to create a less heterogeneous enamel substrate and may therefore be expected to show more predictable lesion behaviour. However, this was not evident here when considering the correlation between DIAGNOdent and QLF analysis.

Other workers have assessed the agreement between different fluorescence techniques. Al-Khateeb et al., (1997a) reported a comparison of two different fluorescence methods. This study also used artificial lesions created in both human

and bovine enamel. These lesions were analysed using both the QLF\clin used here and an earlier version of this device which employed an argon-ion laser light source in place of the filtered arc lamp. Al-Khateeb et al., (1997a) demonstrated correlation of the two individual techniques with mineral loss as measured by microradiography and in addition assessed the correlation between the two methods of analysis. Pearson's correlation coefficient was calculated as 0.93 indicating a high degree of correlation. However, the principles of operation and details of construction of the two methods under test were very similar and a high degree of agreement between the two was not unexpected. In contrast, the DIAGNOdent and QLF\clin have considerable technical differences and this is reflected in the far lower correlation between the two devices demonstrated here.

Tranæus et al., (2000) also compared the DIAGNOdent and another QLF device for detection and quantification of caries on smooth surfaces. Non-cavitated smooth surface carious lesions from extracted premolar teeth were analysed by the two methods. In addition both histological and TMR analyses were carried out on sections from each lesion to determine lesion depth and mineral loss which served as the gold standards. Pearson's correlation coefficients between mineral loss and the two test methods were calculated and found to be 0.69 for QLF and 0.58 for DIAGNOdent. The correlation of QLF with the gold standard was similar to that found in this study but the value of 0.58 for DIAGNOdent correlation is higher than was demonstrated here. No direct correlation between the two methods was calculated by Tranæus et al., (2000) but the authors concluded that QLF was superior

to DIAGNOdent analysis for smooth surface caries diagnosis. In this respect, their findings are similar to those discussed here.

CHAPTER 3

THE INFLUENCE OF EXOGENOUS STAIN ON LESION ANALYSIS WITH DIAGNOdent AND QLF \ clin

3.1 DIAGNOdent Analysis of Staining Materials: A Preliminary Investigation.

3.1.1 Introduction

It was noted during use of the DIAGNOdent that the subjective degree of discolouration within the lesion being analysed may have some bearing on the subsequent DIAGNOdent value obtained. In order to study this phenomenon in laboratory created carious lesions, it was first necessary to develop a suitable protocol for artificially introducing stain into the lesions. Clinical experience suggests the potential for staining of restorations and demineralised lesions by certain foods and drinks and a short pilot study was proposed to help select the most suitable materials for modelling this effect.

3.1.2 Materials and Methods

A number of potentially stain producing substances were investigated for their fluorescence as measured by the DIAGNOdent. The materials studied were:

- Tea
- Coffee
- Coca-Cola
- Red wine
- Corsodyl mouthwash (chlorhexidine gluconate 0.2%)
- Water (control)

A standard solution of tea was produced by placing one tea bag into 200ml of boiling water and stirring with a magnetic stirrer for 3 minutes. Similarly a solution of coffee was produced with one teaspoonful of instant coffee dissolved in 200 ml of water. Proprietary brands of the other materials were used in their unaltered states as supplied by the manufacturers.

0.5ml of each material was dropped onto a clean filter paper and allowed to dry in air for 2 hours. The DIAGNOdent device was given a baseline reference point on a clean dry area of the filter paper before passing the probe tip over the test area. The probe position and angulation was varied and the peak value for each material recorded.

3.1.3 Results

The peak DIAGNOdent values recorded for each material are shown in table 3.1.

Table 3.1. Peak DIAGNOdent Values for Staining Materials

Material	Peak DIAGNOdent Value
Water	0
Corsodyl Mouthwash	0
Tea	99
Coffee	28
Red Wine	27
Coca-Cola	4

3.1.4 Discussion

The results showed that the DIAGNOdent device was insensitive to both water and chlorhexidene. Coca-Cola, coffee and tea all produced a response, with tea resulting in the greatest amount of detectable fluorescence. Tea was therefore was chosen as the most suitable staining material for further studies into staining phenomena.

3.2 The Influence of Exogenous Stain on DIAGNOdent Analysis of Artificially Created Carious Lesions: A Pilot Study.

3.2.1 Introduction

Section 3.1 has described the DIAGNOdent detection of fluorescence produced by staining materials when applied to clean, dry filter paper. The effect of the same staining materials on the DIAGNOdent analysis of artificially created carious lesions, however was unknown. A pilot study to investigate this effect was undertaken.

3.2.2 Materials and Methods

3.2.2.1 Lesion Creation

Twenty teeth with intact buccal surfaces were selected from a pool of extracted teeth. These were cleaned and pellicle removed with a rotating bristle brush and pumice. The teeth were then bisected bucco-lingually and varnished to produce bare enamel windows on the buccal aspect in the same manner as described in section 2.2.2.

The resulting 40 specimens were subjected to demineralisation in 'Carbopol' solution for 96 hours. On completion the varnish was removed as described previously (section 2.2.5) and the specimens stored in a humid environment for subsequent analysis.

DIAGNOdent analysis of these lesions were undertaken in the same way as described in section 2.2.7 in order to obtain baseline pre-stain readings for these specimens.

3.2.2.2 Staining of Lesions

A standard tea solution was prepared as in section 3.1.2 by placing a tea bag in 200ml of recently boiled water and stirring with a magnetic stirrer for 3 minutes. All specimens were placed into individual screw top containers containing 10ml of the tea solution. These 40 specimens were then divided into 5 groups of 8 and each

group allocated one of 5 different staining periods namely 6, 12, 24, 48 and 72 hours. During staining, specimens were stored at 37 degrees centigrade and at the end of the prescribed period, specimens from each group were removed, washed with distilled water and placed into labelled screw cap sample bottles for subsequent analysis.

The stained specimens were analysed with the DIAGNOdent in an identical manner to that used prior to staining.

3.2.3 Results

The mean DIAGNOdent value for all lesions was calculated for the pre and post staining analysis. The mean pre-stain DIAGNOdent value was 1.15 and the mean post stain value was 22.23. This shows a substantial increase in the DIAGNOdent value following exposure to the staining solution. The mean DIAGNOdent value for all lesions for the individual staining time periods was also calculated for the pre and post staining DIAGNOdent analyses. The Wilcoxon Signed Ranks Test was used to determine the significance of the observed change in DIAGNOdent values following exposure to the staining protocol. Results are shown in table 3.2.

Table 3.2. Mean DIAGNOdent Value According to Staining Time

Staining Time (Hours)	Pre-Stain Mean DIAGNOdent Value (S.E.M.)	Post-Stain Mean DIAGNOdent Value (S.E.M.)	Wilcoxon Signed Ranks Test p-value
6	1.00 (0.27)	1.50 (0.42)	0.36
12	1.00 (0.50)	1.63 (0.46)	0.06
24	1.13 (0.44)	21.88 (7.66)	0.01
48	1.38 (0.26)	45.25 (10.73)	0.01
72	1.25 (0.25)	40.88 (12.04)	0.01

3.2.4 Discussion

Pre-staining analysis failed to demonstrate any marked degree of fluorescence as indicated by a raised DIAGNOdent value. This is in agreement with the results in section 2.3.1 where 96 hour 'Carbopol' lesions demonstrated a mean DIAGNOdent value of 1.09. The values obtained during analysis of these lesions was similar.

A prominent increase in recorded DIAGNOdent value was seen following exposure to the staining tea solution. However, this effect varied depending on the length of time for which the specimens were exposed to the solution. The 6 and 12 hour stained lesions did not exhibit visible staining of either the lesion or the surrounding tooth surface. The recorded DIAGNOdent values for these lesions were slightly increased but this was not significant ($p>0.05$). Lesions exposed to the staining solution for 24, 48 and 72 hours demonstrated visible staining of the lesion (figure 3.1) as well as significant increases in the post-staining DIAGNOdent values.($p=0.01$). However, the 48 and 72 hour groups exhibited generalised staining of the whole tooth surface beyond the confines of the artificial lesion (figure 3.2).

Fig. 3.1 Stained Demineralised Lesion

Fig. 3.2 Staining of Whole Tooth

It was concluded that exposure to a staining solution had influenced the DIAGNOdent analysis of these artificially created carious lesions. The differing results of the 5 staining time periods suggested that for further studies, 24 hours was the most appropriate period for staining exposure since this period brought about a significant influence on DIAGNOdent analysis without excessive staining beyond the confines of the lesion.

3.3 The Influence of Exogenous Stain on Artificially Created Carious Lesions

3.3.1 Introduction

Results from section 3.2 suggested the modification of DIAGNOdent analysis of artificial carious lesions by the introduction into the lesion of exogenous stain. Another study was proposed to investigate this further and to determine the effect of lesion staining on the observed ability of the DIAGNOdent to discriminate varying demineralisation times as determined in Chapter 2.

3.3.2 Materials and Methods

3.3.2.1 Specimen Creation

The existing 220 tooth specimens from the experimental work described in Chapter 2 were used for this study. In individual 10ml vials, each specimen was immersed for 24 hours in 10ml of standard tea solution. Following the staining period, the teeth and vials were washed with distilled water and the teeth placed back into their sample bottles with a moist thymol cotton pledget to create a humid storage atmosphere.

3.3.2.2 Specimen Analysis

DIAGNOdent and QLF analyses of the stained specimens were carried out in the same way as for the unstained specimens described in section 2.2.7. The analysis was carried out by 4 different examiners, at different times, yielding four separate sets of results for each specimen. DIAGNOdent analysis was undertaken twice for each specimen yielding a total of 8 results for each lesion.

3.3.3 Results

The distribution of the data was examined and the Shapiro-Wilk statistic was calculated to determine whether or not the data were normally distributed. Results indicated that the data were extremely skewed and therefore non-parametric statistical tests were used.

3.3.3.1 DIAGNOdent Analysis

The mean and median DIAGNOdent values for each demineralisation time period were calculated for each of the four examiners. Results are shown in tables 3.3-3.6.

Table 3.3. Mean and Median DIAGNOdent Values for Each Demineralisation Time (Examiner 1)

Demineralisation Time (Hours)	Mean DIAGNOdent Value (S.E.M.)		Median DIAGNOdent Value	
	Reading I	Reading II	Reading I	Reading II
0	0.55 (0.14)	0.35 (0.13)	0.50	0.00
8	3.08 (0.55)	3.13 (0.42)	2.00	2.00
24	6.48 (0.88)	6.48 (0.77)	5.00	6.00
48	9.75 (1.34)	10.13 (1.42)	10.00	9.50
72	11.05 (2.10)	10.18 (1.71)	7.00	7.00
96	16.70 (3.58)	16.38 (3.39)	10.50	9.00

Table 3.4. Mean and Median DIAGNOdent Values for Each Demineralisation Time (Examiner 2)

Demineralisation Time (Hours)	Mean DIAGNOdent Value (S.E.M.)		Median DIAGNOdent Value	
	Reading I	Reading II	Reading I	Reading II
0	0.45 (0.11)	0.30 (0.11)	0.00	0.00
8	3.10 (0.44)	3.28 (0.45)	2.00	2.00
24	6.15 (0.85)	5.83 (0.79)	4.00	5.00
48	10.40 (1.43)	10.10 (1.41)	9.00	10.50
72	10.53 (1.65)	10.68 (1.72)	6.50	7.50
96	17.43 (3.96)	16.27 (3.36)	10.00	9.50

Table 3.5. Mean and Median DIAGNOdent Values for Each Demineralisation Time (Examiner 3)

Demineralisation Time (Hours)	Mean DIAGNOdent Value (S.E.M.)		Median DIAGNOdent Value	
	Reading I	Reading II	Reading I	Reading II
0	2.35 (2.19)	2.75 (2.28)	0.00	0.00
8	2.15 (0.39)	2.20 (0.35)	1.00	2.00
24	2.70 (0.41)	2.40 (0.41)	2.00	2.00
48	4.00 (0.57)	4.10 (0.60)	3.00	3.00
72	3.80 (0.65)	4.00 (0.78)	2.00	1.50
96	5.95 (1.44)	5.40 (1.21)	3.50	4.00

Table 3.6. Mean and Median DIAGNOdent Values for Each Demineralisation Time (Examiner 4)

Demineralisation Time (Hours)	Mean DIAGNOdent Value (S.E.M.)		Median DIAGNOdent Value	
	Reading I	Reading II	Reading I	Reading II
0	0.50 (0.31)	0.65 (0.26)	0.00	0.00
8	1.95 (0.31)	1.60 (0.29)	2.00	1.00
24	2.40 (0.32)	2.38 (0.32)	2.00	2.50
48	3.78 (0.65)	3.38 (0.57)	3.00	2.00
72	3.25 (0.65)	3.45 (0.63)	1.50	2.00
96	4.47 (0.87)	4.60 (1.04)	3.50	3.00

3.3.3.2 Intra-Examiner Agreement for DIAGNOdent Analysis

The correlation between the first and second DIAGNOdent analyses carried out for each specimen was calculated for each of the four examiners. Results are shown in table 3.7.

Table 3.7. Intra-Examiner Correlation for DIAGNOdent Analysis

Examiner	Spearman's Correlation Coefficient (Reading 1 vs Reading 2)	p value
1	0.94	0.000
2	0.94	0.000
3	0.90	0.000
4	0.86	0.000

3.3.3.3 Inter-Examiner Agreement for DIAGNOdent Analysis

The correlation of DIAGNOdent analysis of specimens between different examiners was calculated and significance determined. In addition, the Wilcoxon Signed Ranks Test was used to determine the existence of significant differences between the results of the individual examiners. Results are shown in table 3.8.

Table 3.8. Inter-Examiner Correlation for DIAGNOdent Analysis

Examiners	Spearman's Correlation Coefficient (p value)	Wilcoxon Signed Ranks Test p value
1 vs 2	0.93 (0.000)	0.886
1 vs 3	0.69 (0.000)	0.000
1 vs 4	0.67 (0.000)	0.000
2 vs 3	0.68 (0.000)	0.000
2 vs 4	0.64 (0.000)	0.000
3 vs 4	0.66 (0.000)	0.077

3.3.3.4 Correlation Of DIAGNOdent Analysis With Demineralisation Time

The correlation between DIAGNOdent value and demineralisation time was calculated for all lesions irrespective of lesion creation protocol (surface abrasion and demineralisation solution). As there appeared to be interactions between the type of demineralisation solution and the use of surface abrasion, the influence of the different lesion creation protocols was studied and correlations for individual groups on the basis of surface abrasion and demineralisation solution type were also determined. The Kruskal Wallis test was used to determine the existence of any significant differences between groups on the basis of lesion creation protocol. Results are shown in tables 3.9-3.12.

Table 3.9. Correlation of DIAGNOdent Analysis with Demineralisation Time for Different Lesion Creation Protocols (Examiner 1)

Lesion Group	Number of Lesions	r^s (p Value)	KW p value
All	220	0.47 (0.000)	0.000
Abraded / 'Carbopol'	60	0.83 (0.000)	0.000
Abraded / 'Glasgow'	60	0.61 (0.000)	0.000
Unabraded / 'Carbopol'	60	0.57 (0.000)	0.002
Unabraded / 'Glasgow'	60	0.39 (0.002)	0.015

Table 3.10. Correlation of DIAGNOdent Analysis with Demineralisation Time for Different Lesion Creation Protocols (Examiner 2)

Lesion Group	Number of Lesions	r^s (p Value)	KW p value
All	220	0.48 (0.000)	0.000
Abraded / 'Carbopol'	60	0.85 (0.000)	0.000
Abraded / 'Glasgow'	60	0.57 (0.000)	0.000
Unabraded / 'Carbopol'	60	0.64 (0.000)	0.000
Unabraded / 'Glasgow'	60	0.51 (0.000)	0.001

Table 3.11. Correlation of DIAGNOdent Analysis with Demineralisation Time for Different Lesion Creation Protocols (Examiner 3)

Lesion Group	Number of Lesions	r^s (p Value)	KW p value
All	220	0.30 (0.000)	0.000
Abraded / 'Carbopol'	60	0.62 (0.000)	0.000
Abraded / 'Glasgow'	60	0.50 (0.000)	0.002
Unabraded / 'Carbopol'	60	0.28 (0.029)	0.009
Unabraded / 'Glasgow'	60	0.32 (0.014)	0.146

Table 3.12. Correlation of DIAGNOdent Analysis with Demineralisation Time for Different Lesion Creation Protocols (Examiner 4)

Lesion Group	Number of Lesions	r^s (p Value)	KW p value
All	220	0.31 (0.000)	0.000
Abraded / 'Carbopol'	60	0.57 (0.000)	0.001
Abraded / 'Glasgow'	60	0.59 (0.000)	0.000
Unabraded / 'Carbopol'	60	0.30 (0.018)	0.258
Unabraded / 'Glasgow'	60	0.24 (0.069)	0.232

3.3.3.5 QLF Analysis

The mean and median QLF parameters (mean loss of fluorescence, maximum loss of fluorescence and lesion area) for all specimens, irrespective of demineralising solution or enamel surface preparation according to demineralisation time were calculated for each examiner. Results are shown in tables 3.13-3.20.

Table 3.13. Mean QLF Parameters for all Lesions (Examiner 1)

Demin Time (Hours)	ΔL_{mean} (%) (S.E.M.)	ΔL_{max} (%) (S.E.M)	Lesion Area (mm^2) (S.E.M.)
0	-11.62 (0.46)	-18.95 (1.48)	0.09 (0.03)
8	-13.60 (0.41)	-32.15 (2.21)	1.79 (0.32)
24	-20.86 (1.09)	-47.38 (2.64)	3.19 (0.37)
48	-25.19 (1.52)	-52.35 (2.72)	3.99 (0.39)
72	-25.85 (1.58)	-54.63 (2.64)	4.63 (0.36)
96	-30.62 (1.95)	-60.08 (2.59)	5.28 (0.43)

Table 3.14. Median QLF Parameters for all Lesions (Examiner 1)

Demin Time (Hours)	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
0	-10.85	-17.00	0.00
8	-13.30	-30.50	1.15
24	-20.05	-48.00	3.25
48	-25.50	-54.50	3.75
72	-25.05	-57.00	4.75
96	-30.65	-61.50	5.35

Table 3.15. Mean QLF Parameters for all Lesions (Examiner 2)

Demin Time (Hours)	ΔL_{mean} (%) (S.E.M.)	ΔL_{max} (%) (S.E.M)	Lesion Area (mm^2) (S.E.M.)
0	-11.34 (0.29)	-18.40 (1.78)	0.13 (0.07)
8	-13.49 (0.41)	-32.48 (2.45)	1.60 (0.26)
24	-20.95 (1.24)	-46.95 (2.90)	3.37 (0.40)
48	-25.75 (1.62)	-52.38 (2.59)	4.29 (0.47)
72	-26.23 (1.64)	-54.98 (2.63)	4.76 (0.45)
96	-31.89 (1.87)	-63.23 (2.23)	5.33 (0.42)

Table 3.16. Median QLF Parameters for all Lesions (Examiner 2)

Demin Time (Hours)	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
0	-10.80	-14.50	0.00
8	-12.75	-30.00	0.90
24	-20.05	-47.00	3.15
48	-24.15	-54.50	3.70
72	-24.15	-59.00	4.80
96	-31.15	-64.50	5.75

Table 3.17. Mean QLF Parameters for all Lesions (Examiner 3)

Demin Time (Hours)	ΔL_{mean} (%) (S.E.M.)	ΔL_{max} (%) (S.E.M)	Lesion Area (mm^2) (S.E.M.)
0	-13.95 (1.67)	-24.15 (3.64)	0.56 (0.25)
8	-15.51 (1.10)	-36.03 (2.60)	1.93 (0.36)
24	-17.58 (1.08)	-40.20 (2.52)	2.40 (0.29)
48	-20.75 (1.33)	-47.00 (2.82)	3.54 (0.40)
72	-20.51 (1.20)	-48.20 (2.56)	3.68 (0.43)
96	-21.68 (1.59)	-51.28 (2.41)	3.78 (0.37)

Table 3.18. Median QLF Parameters for all Lesions (Examiner 3)

Demin Time (Hours)	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
0	-11.20	-17.50	0.10
8	-12.70	-32.50	1.15
24	-16.50	-41.00	2.20
48	-18.75	-48.00	3.55
72	-18.95	-50.00	3.90
96	-20.10	-52.00	4.00

Table 3.19. Mean QLF Parameters for all Lesions (Examiner 4)

Demin Time (Hours)	ΔL_{mean} (%) (S.E.M.)	ΔL_{max} (%) (S.E.M)	Lesion Area (mm^2) (S.E.M.)
0	-13.30 (1.39)	-23.10 (2.93)	0.28 (0.16)
8	-13.71 (0.41)	-32.03 (1.93)	1.30 (0.18)
24	-15.61 (0.79)	-33.40 (2.40)	1.56 (0.23)
48	-19.03 (1.43)	-39.93 (3.00)	2.03 (0.28)
72	-18.28 (1.19)	-40.35 (2.77)	2.48 (0.31)
96	-20.18 (1.76)	-43.43 (3.09)	2.77 (0.35)

Table 3.20. Median QLF Parameters for all Lesions (Examiner 4)

Demin Time (Hours)	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
0	-11.85	-19.00	0.05
8	-12.95	-30.50	1.05
24	-13.60	-33.00	1.40
48	-16.60	-40.00	1.70
72	-15.65	-38.50	2.20
96	-16.85	-41.00	2.95

3.3.3.6 Inter-Examiner Agreement for QLF Analysis

The correlation of QLF analysis of specimens between different examiners was calculated. In addition, the Wilcoxon Signed Ranks Test was used to determine the existence of significant differences between the results of the individual examiners Results are shown in tables 3.21-3.23.

Table 3.21. Inter-Examiner Correlation for ΔL_{mean} (%)

Examiners	Spearman's Correlation Coefficient (p value)	Wilcoxon Signed Ranks Test p value
1 vs 2	0.92 (0.000)	0.289
1 vs 3	0.54 (0.000)	0.000
1 vs 4	0.60 (0.000)	0.000
2 vs 3	0.58 (0.000)	0.000
2 vs 4	0.61 (0.000)	0.000
3 vs 4	0.73 (0.000)	0.000

Table 3.22. Inter-Examiner Correlation for ΔL_{max} (%)

Examiners	Spearman's Correlation Coefficient (p value)	Wilcoxon Signed Ranks Test p value
1 vs 2	0.89 (0.000)	0.355
1 vs 3	0.53 (0.000)	0.000
1 vs 4	0.56 (0.000)	0.000
2 vs 3	0.60 (0.000)	0.000
2 vs 4	0.58 (0.000)	0.000
3 vs 4	0.73 (0.000)	0.000

Table 3.23. Inter-Examiner Correlation for Lesion Area (mm^2)

Examiners	Spearman's Correlation Coefficient (p value)	Wilcoxon Signed Ranks Test p value
1 vs 2	0.85 (0.000)	0.670
1 vs 3	0.61 (0.000)	0.000
1 vs 4	0.59 (0.000)	0.000
2 vs 3	0.62 (0.000)	0.000
2 vs 4	0.64 (0.000)	0.000
3 vs 4	0.80 (0.000)	0.000

3.3.3.7 Correlation of QLF Analysis with Demineralisation Time

The correlation between QLF parameter values and demineralisation time was calculated for all lesions irrespective of lesion creation protocol (surface abrasion and demineralisation solution). As there appeared to be interactions between the type of demineralisation solution and the use of surface abrasion, the influence of the different lesion creation protocols was studied and correlations for individual groups on the basis of surface abrasion and demineralisation solution type were also determined. The Kruskal Wallis test was used to determine the existence of any significant differences between groups on the basis of lesion creation protocol. Results are shown in tables 3.24-3.26.

Table 3.24. Correlation of ΔL_{mean} (%) with Demineralisation Time for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s (p Value)	KW p value
All	220	-0.41 (0.000)	0.000
Abraded / 'Carbopol'	60	-0.63 (0.000)	0.000
Abraded / 'Glasgow'	60	-0.65 (0.000)	0.000
Unabraded / 'Carbopol'	60	-0.42 (0.001)	0.026
Unabraded / 'Glasgow'	60	-0.42 (0.066)	0.136

Table 3.25. Correlation of ΔL_{max} (%) with Demineralisation Time for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s (p Value)	KW p value
All	220	-0.42 (0.000)	0.000
Abraded / 'Carbopol'	60	-0.66 (0.000)	0.000
Abraded / 'Glasgow'	60	-0.69 (0.000)	0.000
Unabraded / 'Carbopol'	60	-0.40 (0.001)	0.044
Unabraded / 'Glasgow'	60	-0.31 (0.016)	0.080

Table 3.26. Correlation of Lesion Area (mm^2) with Demineralisation Time for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s (p Value)	KW p value
All	220	0.42 (0.000)	0.000
Abraded / 'Carbopol'	60	0.80 (0.000)	0.000
Abraded / 'Glasgow'	60	0.73 (0.000)	0.000
Unabraded / 'Carbopol'	60	0.37 (0.003)	0.065
Unabraded / 'Glasgow'	60	0.21 (0.100)	0.136

3.3.3.8 Correlation of DIAGNOdent Analysis with QLF Analysis

Correlations between DIAGNOdent analysis and the three measured QLF parameters (ΔL_{mean} (%), ΔL_{max} (%), and Lesion Area (mm^2)) for all lesions and individual groups based on lesion creation protocol were calculated. Results are shown in table 3.27-3.29.

Table 3.27. Correlation of DIAGNOdent Values with ΔL_{mean} (%) for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s	p value
All	220	-0.42	0.000
Abraded / 'Carbopol'	60	-0.68	0.000
Abraded / 'Glasgow'	60	-0.56	0.000
Unabraded / 'Carbopol'	60	-0.46	0.000
Unabraded / 'Glasgow'	60	-0.09	0.520

Table 3.28. Correlation of DIAGNOdent Value with ΔL_{max} (%) for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s	p value
All	220	-0.46	0.000
Abraded / 'Carbopol'	60	-0.70	0.000
Abraded / 'Glasgow'	60	-0.67	0.000
Unabraded / 'Carbopol'	60	-0.47	0.000
Unabraded / 'Glasgow'	60	-0.13	0.320

Table 3.29. Correlation of DIAGNOdent Value with Lesion Area (mm^2) for Different Lesion Creation Protocols

Lesion Group	Number of lesions	r^s	p value
All	220	0.56	0.000
Abraded / 'Carbopol'	60	0.67	0.000
Abraded / 'Glasgow'	60	0.54	0.000
Unabraded / 'Carbopol'	60	0.48	0.000
Unabraded / 'Glasgow'	60	0.08	0.569

3.3.4 Discussion

The effect of exogenous stain on either DIAGNOdent or QLF analysis of demineralised lesions has not been investigated or discussed previously. The clinical importance of this effect is the possibility that contact of carious lesions with chromogenic food substances (e.g. tea) has the potential to influence the DIAGNOdent or QLF assessment of these lesions. This may theoretically lead to inappropriate clinical management of the lesion, in particular unwarranted operative intervention or restoration of a clinically suspect lesion.

3.3.4.1 Lesion Staining

Tea has been investigated in respect of its mineral content and has been shown to contain high concentrations of potassium, calcium, magnesium, sulphur, aluminium, fluoride, manganese and iron as well as trace amounts of nickel, cobalt, zinc, copper, lead and cadmium (Spiers 1983). However, it seems likely that it is the organic constituents of tea which are responsible for the observed fluorescence when tea stains are analysed by DIAGNOdent and QLF. No analysis of the organic components of tea ash has so far been undertaken and the specific compounds responsible have not been identified.

In the experimental work undertaken here, specimens were visibly stained when removed from the tea staining solution. The staining protocol followed involving 24

hours staining period was developed as a result of the pilot studies described in sections 3.1 and 3.2. Staining for periods longer than 24 hours resulted in discolouration of areas of sound tissue surrounding the lesion area and this led to an altered DIAGNOdent reference value for sound tissue. It was considered important to attempt to preserve the sound reference site unaltered to allow comparison between unstained and stained specimens. Therefore, 24 hours exposure to staining allowed introduction of stain into the lesion without alteration of the surrounding sound tooth tissue.

The protocol for lesion analysis followed for the unstained specimens in section 2.2.7.1 was repeated for the stained specimens. Thus, consecutive DIAGNOdent analyses of each specimen were carried out by each of the 4 examiners yielding a total of 8 results for each specimen.

3.3.4.2 Intra-Examiner Agreement

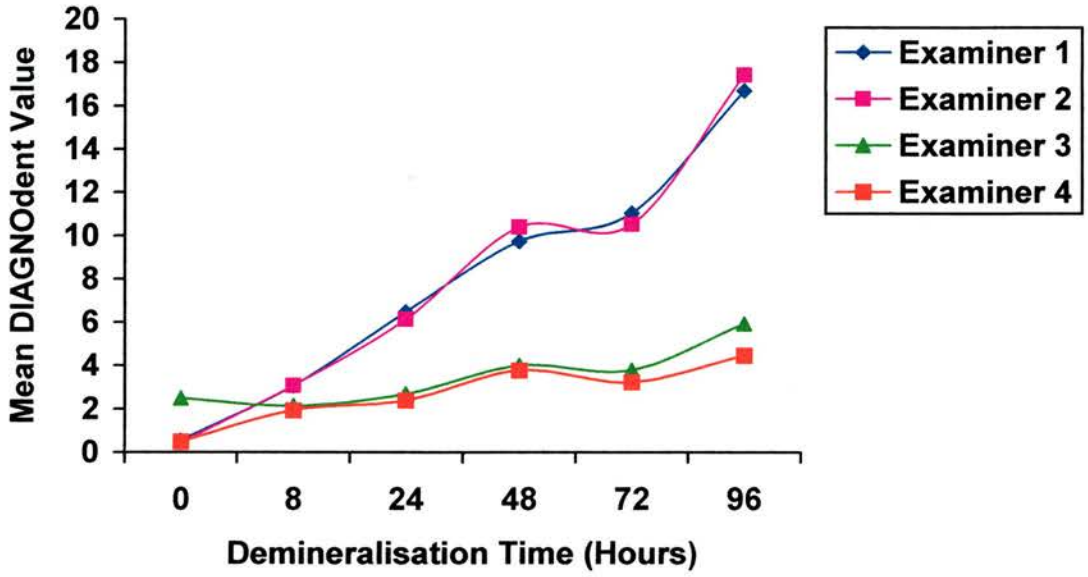
The 2 separate analyses of each specimen carried out by each examiner were subjectively similar and consideration was given to the handling of these paired results. Calculation of Spearman's correlation coefficient for the two analyses (table 3.7) indicated highly significant correlations ($p<0.001$) between the two readings for all examiners and it was therefore considered appropriate to use only the first data set for each examiner in subsequent analyses. This allowed considerably more straightforward data analysis.

3.3.4.3 Inter-Examiner Agreement

Results appeared subjectively similar for each of the 4 individual examiners and inter-examiner agreement was tested by calculating Spearman's correlation coefficient for all possible examiner pairs. Results indicated highly significant correlations between each examiner pair (table 3.8).

However, subjective analysis of results was suggestive of a difference between the DIAGNOdent values of examiners 1 and 2 and examiners 3 and 4. Availability of the 4 individual examiners for lesion analysis resulted in examiners 1 and 2 carrying out their analysis on the same day (immediately following removal of specimens from the staining solution) and examiners 3 and 4 carrying out their analysis 6 and 7 days later respectively. Although the Spearman's correlation coefficients of all the possible examiner pairs were statistically significant, the Wilcoxon signed ranks test results comparing the results from each examiner indicated statistically significant differences ($p<0.001$) between examiners 1 and 2 and examiners 3 and 4. By contrast, no significant differences were demonstrated between 1 and 2 or 3 and 4 (table 3.8). A plot of mean DIAGNOdent value against demineralisation time for each examiner illustrates the apparent difference exerted by the timing of the analysis (figure 3.3).

Figure 3.3. Plot of Mean DIAGNOdent Value against Demineralisation Time for Individual Examiners



In comparison, with the exception of a single examiner pair, analysis of the same specimens by an identical protocol and by the same individuals *prior to staining*, indicated no significant differences between examiners (table 2.6). It was postulated therefore, that the different elapsed time between staining and analysis for examiners 1 and 2 in comparison with examiners 3 and 4 may have influenced their respective results and it was therefore considered important to investigate the observed relationship between demineralisation time and DIAGNOdent value for all 4 individual examiners (section 3.3.4.4).

The observed difference between the results of the DIAGNOdent analyses carried out at different times following staining suggested that adsorption of stain into the demineralised lesion may not be stable over a prolonged period and may 'decay' or wash out of the lesion. Specimens were stored in a sealed, humid environment in individual containers in the period between staining and analysis but nevertheless, the staining effect was apparently unstable. This phenomenon required further investigation and additional work undertaken to do this is described in section 3.4.

3.3.4.4 Correlation of DIAGNOdent Value with Demineralisation Time

As for unstained specimens (tables 2.1-2.4), the DIAGNOdent values for all stained lesions, irrespective of the demineralising protocols of the study, were examined in relation to the demineralisation time period. Subjective analysis of calculated mean and median values for DIAGNOdent analysis indicated a definite trend of increasing DIAGNOdent value with increasing demineralisation time (tables 3.3-3.6). However, as discussed in 3.3.4.3, this simple preliminary analysis of means and medians indicated a difference in the trend between examiners 1 and 2 and examiners 3 and 4.

Statistical analysis of DIAGNOdent values in relation to demineralisation time for all lesions did indicate a significant correlation between these parameters (table 3.9-3.12). For all 220 specimens, regardless of lesion creation protocol, the Spearman's correlation co-efficient ranged between 0.30 and 0.48 ($p<0.001$). Although all examiners were able to demonstrate a significant correlation, it was noted that the degree of correlation varied between examiners 1 and 2 and examiners 3 and 4. This appeared to add to the theory of instability in the influence of staining on the lesions. As was the case for the unstained lesions analysed in the same way and discussed in section 2.4.3.3, the relatively low values for correlation did not indicate a close relationship between DIAGNOdent value and demineralisation time although the large sample sizes did result in a high degree of significance for these correlations ($p<0.001$).

The Kruskal Wallis test was also used to determine the existence of significant differences in DIAGNOdent values between the different demineralisation times. For all lesions, irrespective of the lesion creation protocol, all examiners demonstrated a highly significant ability ($p<0.001$) of the DIAGNOdent to distinguish between the different exposure times (tables 3.9-3.12). On this basis, these results confirmed the validity of the DIAGNOdent value as a measure of demineralisation of artificial lesions.

3.3.4.5 Influence of Lesion Creation Protocol

The use of the two distinct demineralising protocols with variation in surface preparation and demineralising solution offered the potential for comparison in respect of the fluorescence analysis. Analysis of the relationship between DIAGNOdent value and demineralisation time for all 220 lesions suggested the existence of interactions between the type of demineralisation solution and the surface abrasion used in lesion creation. Therefore, the influence of each separate lesion creation protocol was studied. Tables 3.9-3.12 also show the correlation between DIAGNOdent value and demineralisation time for each individual lesion creation protocol.

The influence of surface abrasion was consistently positive. For both 'Carbopol' and 'Glasgow' created lesions, abraded specimens demonstrated a higher correlation with demineralisation time than unabraded specimens. This was consistent for all 4

examiners. Kruskal Wallis tests to determine significant differences between different demineralisation periods were also performed for each lesion creation protocol and these results were also indicative of a consistently stronger performance of the DIAGNOdent when analysing abraded lesions (tables 3.9-3.12). As discussed previously (2.4.3.4), the effect of removing the outer heterogeneous layer of enamel was expected to result in a more homogeneous and predictable lesion. The results of analysis of these stained artificial lesions appeared to confirm this phenomenon.

The influence of demineralising solution was less consistent but results of Spearman's Correlation Coefficients and Kruskal Wallis tests indicated a stronger relationship between DIAGNOdent value and demineralisation time in 'Carbopol' lesions in comparison to 'Glasgow' lesions (tables 3.9-3.12). With the exception of the unabraded lesions analysed by examiners 3 and 4, analyses for both abraded and unabraded lesions by all examiners suggested more predictable DIAGNOdent behaviour when analysing 'Carbopol' lesions.

This is in contrast to the results indicated by the analyses of the same lesions prior to staining described in 2.4.3.4. In this situation, it was evident that there was no clear effect being exerted by the lesion creation protocol and results did not follow a particular trend according to the method of lesion creation. However, the introduction of stain, into the lesion did appear to show a more consistent difference between the different lesion types. In addition, the correlation between DIAGNOdent value and demineralisation time appeared to be stronger when the same lesions were analysed

after exposure to the staining protocol. In this way it appeared that the introduction of stain into the lesion did have an effect on the performance of the DIAGNOdent device.

3.3.4.6 QLF Analysis

The protocol for QLF analysis of the stained specimens was identical to that for the same specimens prior to staining. Practical considerations in relation to QLF analysis of the unstained lesions were discussed in 2.4.4 and these are also of relevance to the analysis of the stained lesions discussed here.

3.3.4.7 Inter-Examiner Agreement

As for analysis of lesions prior to staining (2.4.4.1), the QLF parameter values appeared subjectively similar for the 4 individual examiners. Inter-examiner agreement for QLF analysis of specimens post-staining was tested by calculating Spearman's correlation coefficient for all possible examiner pairs for each QLF parameter (tables 3.21-3.23). Results indicated highly significant correlations between each examiner pair ($p<0.001$). The Wilcoxon Signed Ranks Test was also used to test for significant differences between the results of each examiner pair (tables 3.21-3.23). With the exception of examiner pair '1 v 2' , results indicated that there were significant differences between examiner pairs. Nevertheless, it was

decided to use the results of the author (examiner 3) in subsequent analyses of QLF data.

As for DIAGNOdent analysis, the practical availability of individuals to carry out the lesion analysis resulted in the 4 examiners undertaking their QLF analysis at different times. The obvious differences between the results of examiners 1 and 2 and examiners 3 and 4 which were apparent for the DIAGNOdent analysis of the specimens (see figure 3.3) was not noted for QLF parameters. Although the mean and median QLF parameter values for 96 hour lesions were lower for examiners 3 and 4 in comparison to examiners 1 and 2, statistical analysis was unable to confirm a significant difference between the results of these individuals.

3.3.4.8 Correlation of QLF Parameters with Demineralisation Time

The QLF parameter values for all lesions irrespective of the different lesion creation protocols were examined in relation to the demineralisation time period. Subjective analysis of calculated mean and median values for ΔL_{mean} (%); ΔL_{max} (%) and Lesion Area (mm^2) indicated a trend of increasing parameter value with increasing demineralisation time (tables 3.13-3.20).

Statistical analysis of QLF parameter values in relation to demineralisation time for all lesions indicated a significant correlation between these parameters and demineralisation time (tables 3.24-3.26). For all 220 specimens, regardless of lesion

creation protocol, the Spearman's correlation co-efficients for the 3 measured parameters were very similar (ΔL_{mean} (%) -0.41; ΔL_{max} (%) -0.42 and Lesion Area (mm^2) 0.42). All three parameters demonstrated highly significant correlations with demineralisation time ($p<0.001$). The Kruskal Wallis test was also used to determine the presence of significant differences between the groups based on demineralisation time and results were highly significant ($p<0.001$) for the ability of all 3 measured QLF parameters to reflect the varying demineralisation time (tables 3.24-3.26).

The degree of correlation with demineralisation time was, for all three QLF parameters, lower than that observed following analysis of the same specimens prior to staining (tables 2.19-2.21). This is in contrast to the results of DIAGNOdent analysis of the same specimens in which the correlation with demineralisation time was seen to improve following lesion staining.

3.3.4.9 Influence of Lesion Creation Protocol

The influence of lesion creation protocol on DIAGNOdent analysis of stained specimens was discussed in 3.3.4.5. The effect of lesion creation protocol on QLF analysis was also investigated and the correlation with demineralisation time was calculated for individual groups based on demineralising solution and use of surface abrasion (table 3.24-3.26). The degree of correlation between demineralisation time and QLF parameters varied according to lesion creation protocol. Unabraded, 'Glasgow' created lesions failed to demonstrate significant correlation for both ΔL_{mean}

(%) and Lesion Area (mm^2) but all other lesion types showed significant correlations for all QLF parameters. In all cases, surface abrasion was associated with a noticeably stronger correlation than unabraded specimens. The effect of demineralisation solution was less evident with no clearly perceivable difference between 'Carbopol' and 'Glasgow' created lesions. This confirms the effect of surface abrasion of enamel prior to lesion creation which was also noted following DIAGNOdent analysis of stained specimens (3.3.4.5) but is in contrast to the observed effects of lesion creation protocol following QLF analysis of the unstained specimens in which there was no obvious consistent influence from either surface abrasion or demineralising solution (2.4.4.3).

3.3.4.10 Correlation between DIAGNOdent and QLF Analysis

Significant correlations between DIAGNOdent and QLF analysis were found for all measured QLF parameters (tables 3.27-3.29). Although statistically significant, correlation values were not high and the agreement between the two techniques did not appear to be strong. In addition, marked differences in the degree of correlation were noted when individual groups were analysed on the basis of lesion creation protocol. For all three QLF parameters, significant correlations ($p<0.001$) were observed for all lesion creation protocols except 'Unabraded / Glasgow'. As with the correlation of DIAGNOdent and QLF parameter values with demineralisation time (see 3.3.4.5 and 3.3.4.9 respectively), analysis of specimens having undergone surface abrasion was associated with stronger correlation between the two

techniques. However, the influence of varying the demineralising solution was not associated with a significant difference in correlation between the two analysis techniques.

This appears to further agree with the previously discussed (2.4.3.4) observation of more predictable behaviour of lesions created in surface abraded enamel. This may be due to the removal of the heterogeneous, environmentally altered outer layer of enamel. This influence of surface abrasion was not apparent when investigating the correlation between DIAGNOdent and QLF parameter values in unstained specimens (section 2.4.5). However, given the less predictable results of DIAGNOdent analysis of these lesions, it seems likely that it was the unpredictable nature of the DIAGNOdent analysis of these unstained specimens which was responsible for the failure to demonstrate the expected effect of surface abrasion.

3.4 An Investigation into the Stability of Exogenous Staining of Artificially Produced Carious Lesions.

3.4.1 Introduction

In the experiment described in section 3.3, it was observed that the 4 different examiners produced quite different results in their analysis of the stained lesions. This was in contrast with the analysis of the same specimens prior to staining in section 2.2.7.1 when an identical protocol was followed by the same investigators. The only difference for the post staining analysis was the elapsed time between staining and lesion analysis and the possibility that this may have exerted some influence on the results was considered. An additional study was proposed to investigate further the effect of post-staining time on the DIAGNOdent analysis of stained lesions

3.4.2 Materials and Methods

3.4.2.1 Specimen Preparation

Ten permanent molar teeth were selected from a pool of extracted teeth. Selection was made on the basis of large intact buccal surfaces. All existing restorations and caries were removed using a normal dental handpiece and burs. Standard clinical criteria of visual appearance and texture were applied to assess when removal of caries had been completed. The teeth were cleaned with an ultrasonic scaling instrument and rotating bristle brush and oil free prophylactic paste. In order to remove all traces of stain and also the fluoride rich surface zone of enamel, the buccal surfaces were abraded to a depth of approximately 300μm using a depth indexed diamond bur and the surfaces subsequently polished using abrasive disks. The buccal side of the tooth was marked on the root to ease orientation and each tooth was sectioned longitudinally with a rotating diamond disk (Labcut 1010) to yield a total of 20 specimens.

3.4.2.2 Creation of Artificial Lesions

Artificial carious lesions were created on the buccal surfaces of these 20 specimens following the protocol described in section 2.2.2. Each specimen was painted with nail varnish to cover the entire surface except for a window of approximately 3mm by 1mm on the buccal surface. The nail varnish was allowed to dry for 24 hours prior

to placement of the specimens into demineralising solution. 'Carbopol' demineralising solution was used for lesion creation and was prepared as described in section 2.2.3. Specimens were exposed to the demineralising solution in individual vials for a period of 72 hours. Specimens were then removed from the demineralising solution and the nail varnish removed using acetone and a small stiff brush.

3.4.2.3 Analysis of Unstained Lesions

Lesions were analysed in an identical way to that described in section 2.2.7. The specimens were randomly numbered 1 to 20 and dried in a stream of compressed air. After zeroing on sound enamel at the cusp tip, the peak DIAGNOdent value was recorded for the demineralised lesion on the buccal surface of each specimen. The lesions were then further analysed with QLF recording values for ΔL_{max}; ΔL_{mean} and lesion area.

3.4.2.4 Staining of Lesions

A staining solution of tea was prepared by placing one tea bag in 200ml of boiling water and stirring with a magnetic stirrer for 3 minutes. 5 ml of the resulting solution was pipetted into each of 20 Bijou 5ml vials and a single tooth specimen placed into each vial. These were store at 37 degrees centigrade for 24 hours.

3.4.2.5 Analysis of Stained Lesions

After 24 hours the specimens were each removed, dried and DIAGNOdent analysis of the demineralised lesions performed immediately. The specimens were replaced in individual vials with a cotton pledget moistened with PBS. DIAGNOdent analysis was repeated after a further 1, 2, 3, 4, 5, 6, 7, 8, 20 and 27 days. The peak DIAGNOdent value for each lesion was recorded at each time point. QLF analysis of the specimens was carried out after 6, 20 and 27 days following removal from the staining solution and, for each lesion, the 3 QLF parameters (ΔL_{mean} (%); ΔL_{max} (%); Lesion Area (mm^2)) were recorded. In the interim periods, the specimens were stored in a humid buffered environment.

3.4.3 Results

3.4.3.1 DIAGNOdent Analysis

The mean and median DIAGNOdent values for all 20 lesions were calculated for each DIAGNOdent analysis of the specimens. Results are shown in table 3.30.

Table 3.30. Mean and Median DIAGNOdent Values for Each Analysis

Time Point in Relation to Staining	Mean DIAGNOdent Value (S.E.M)	Median DIAGNOdent Value
Pre-stain	1.55 (0.22)	1.00
Immediately Post-stain	30.40 (5.31)	22.00
24 Hours Post-stain	22.15 (5.15)	14.00
48 Hours Post-stain	21.45 (4.32)	15.00
72 Hours Post-stain	17.25 (3.29)	13.00
96 Hours Post-stain	16.30 (3.76)	11.00
5 Days Post-stain	13.20 (2.93)	9.00
6 Days Post-stain	11.00 (2.03)	8.50
7 Days Post-stain	10.15 (2.01)	7.00
8 Days Post-stain	10.75 (2.03)	7.00
20 Days Post-stain	11.15 (1.92)	7.50
27 Days Post-stain	11.05 (2.04)	8.00

A scatter plot of DIAGNOdent values against time in relation to staining was drawn (figure 3.4) and statistical software 'Stata' was used to calculate the regression line for DIAGNOdent value against time elapsed after staining. This method of analysis took account of the repeat measurements of the same specimens over a period of time. Results are shown in table 3.31.

Figure 3.4. Scatter Plot of DIAGNOdent Value against Time Elapsed after Staining

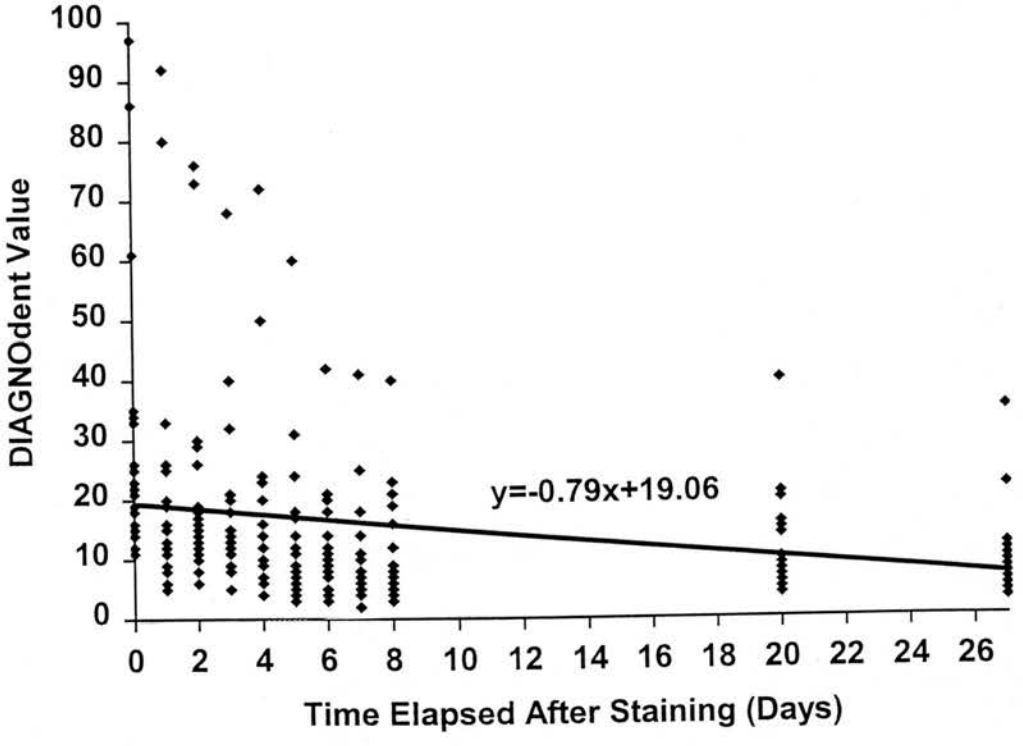


Table 3.31. Results of Regression Analysis with Robust Standard Errors for DIAGNOdent Values in relation to Time Elapsed after Staining (Table Generated by Stata)

	Coefficient	Robust Standard Error	t	p value
Time point	-0.79	0.21	-3.70	<0.002
Constant	19.06	3.72	5.12	<0.000

The test statistic (t) tests the null hypothesis that the true slope of the line is zero i.e. changing time has no effect on the DIAGNOdent value. However, these results indicated that there was, in fact, a significant relationship ($p<0.002$) between the two variables demonstrating that the DIAGNOdent value does indeed vary with time elapsed after exposure to staining.

3.4.3.2 QLF Analysis

Mean and median values for ΔL_{mean} (%); ΔL_{max} (%) and lesion area (mm^2) were calculated for all 20 lesions for each of the 3 QLF analyses of the specimens. Results are shown in tables 3.32 and 3.33.

Table 3.32. Mean QLF Parameter Values for Lesions Pre and Post Staining

Time Elapsed Since Staining	**QLF Parameter**		
	ΔL_{mean} (%) (S.E.M.)	**ΔL_{max} (%) (S.E.M.)**	**Lesion Area (mm^2) (S.E.M.)**
Pre-stain	-28.16 (1.52)	-50.35 (1.98)	4.81 (0.29)
6 Days	-48.12 (1.04)	-76.55 (0.67)	6.65 (0.34)
20 Days	-48.59 (0.96)	-78.00 (0.79)	6.06 (0.29)
27 Days	-45.75 (1.52)	-75.20 (1.31)	6.53 (0.30)

Table 3.33. Median QLF Parameter Values for Lesions Pre and Post Staining

Time Elapsed Since Staining	QLF Parameter		
	ΔL_{mean} (%)	ΔL_{max} (%)	Lesion Area (mm^2)
Pre-stain	-27.80	-51.00	4.55
6 Days	-48.05	-77.00	6.70
20 Days	-48.35	-78.50	6.15
27 Days	-44.35	-75.00	6.50

The Mann-Whitney U test was used to test for significant differences between the pre-stain QLF values and the values at each of the post stain analyses. Results are shown in table 3.34.

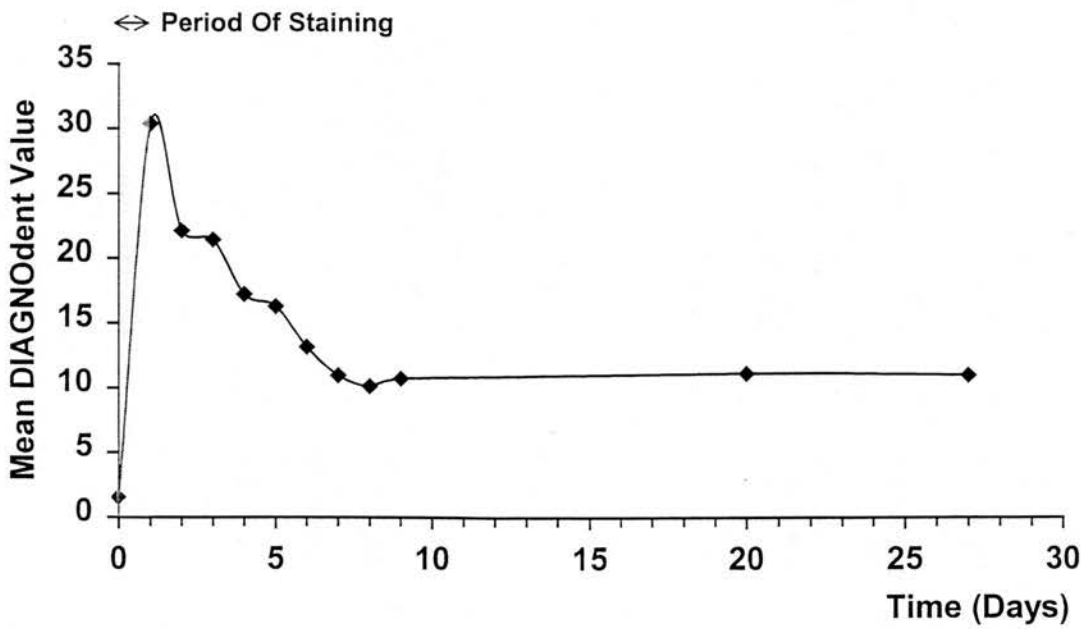
Table 3.34. Mann-Whitney U Test p Values for Comparison of Post Stain QLF Parameters with Pre Stain QLF Parameters

Time Elapsed After Staining	p value ΔL_{mean} (%)	p value ΔL_{max} (%)	p value Lesion Area (mm^2)
6 days	0.000	0.000	0.001
20 days	0.000	0.000	0.005
27 days	0.000	0.000	0.000

3.4.4 Discussion

The unstained 72 hour demineralised lesions created for this experiment failed to stimulate a DIAGNOdent response with all lesions recording a low baseline value below the threshold for clinically significant demineralisation. After staining for 24 hours in tea solution however, all lesions recorded an increased and above threshold value. Over the monitoring period of 27 days however, the mean and median DIAGNOdent values for the lesions were seen to steadily decrease to a new elevated baseline value and the trend for all individual lesions was also for a decrease in DIAGNOdent value. Figure 3.5 illustrates the variation in the mean DIAGNOdent value over the full duration of the experiment.

Figure 3.5 Mean DIAGNOdent Value of Artificial Lesions During Monitoring Period



The results of lesion analysis with DIAGNOdent (3.4.3.1) in this study appear to confirm the observations on the effect of lesion staining made in section 3.3 namely, the uptake of exogenous stain into the lesion was responsible for an increase in DIAGNOdent value. Furthermore, this increase was apparently unstable with regression to a new elevated baseline in 6-7 days. Regression analysis confirmed that the observed instability of the change in DIAGNOdent value was real with results indicating a significant linear relationship between DIAGNOdent value and the time elapsed after exposure to staining. Thus the change in DIAGNOdent value was attributable to a change in time. This is also in agreement with observations discussed in 3.3.4.3 in which differences between the results of post-staining analysis by different examiners was noted with variation attributed to the difference in timing of the analysis by each examiner.

In contrast to DIAGNOdent analysis, QLF analysis was able to detect the unstained lesions but the results of QLF analysis were also subject to change following staining with tea solution. ΔL_{mean} (%); ΔL_{max} (%); and Lesion Area (mm^2) were all increased following exposure of the lesions to the staining solution then apparently stabilising at the new level over the subsequent 27 day monitoring period (tables 3.32-3.33). Unfortunately, due to the unavailability of the QLF\clin apparatus for lesion analysis immediately on removal from the staining solution, the data set for QLF did not allow for regression analysis. Therefore, the Mann-Whitney U test was used to test for significant differences between QLF parameter values at the different time points

after staining. This confirmed highly significant differences for all QLF parameters between unstained and stained lesions (table 3.34).

Due to the absence of QLF parameter data for the time point immediately following the removal of specimens from the staining solution, it is not known whether the observed increase in recorded values was initially larger, thus following the pattern observed in the DIAGNOdent analysis of the same specimens. Section 3.3.4.7 discussed apparent differences between the QLF parameter values obtained by the 4 examiners who carried out their analyses at different times in the previous experiment. However, a real difference brought about by the delay between removal from stain and subsequent analysis was not confirmed by statistical analysis of data. It is similarly not possible to demonstrate a time effect with this data. The more time consuming and less portable nature of QLF \ clin in comparison to DIAGNOdent prevented immediate QLF analysis and thus there remains the unconfirmed possibility of an unstable staining effect on QLF as well as DIAGNOdent analysis

The reason for the observed instability of staining is not clear. A 'leaching out' of adsorbed chromogenic compounds from within the artificial lesion seems the most likely explanation although this has not been investigated previously. Following staining, all specimens were stored in individual vials in buffered, humid conditions but the possibility exists of exchange of compounds between the lesion and its environment. An additional possibility may be an inherent instability of the organic molecules speculated as being responsible for the staining effect of the tea solution

used experimentally. However, the exact nature of these organic compounds is uncertain and therefore information on their behaviour is not known.

The evidence for the validity of artificially created laboratory lesions as models for study in cariology has been discussed in section 1.3. However, the validity of extrapolation of the findings from these studies on staining and their application to the DIAGNOdent analysis of naturally occurring carious lesions is uncertain. The effects of existing organic debris within the lesion have not been accounted for and it was considered appropriate therefore to investigate the potential for altering the DIAGNOdent assessment of caries by the introduction or removal of stain from naturally occurring lesions.

3.5 The Influence of Bleaching and Staining on DIAGNOdent Analysis of Naturally Occurring Carious Lesions

3.5.1 Introduction

Investigations into the effect of exogenous staining on artificial lesions described in sections 3.3 and 3.4 suggested the possibility for influencing the DIAGNOdent analysis of a particular lesion. In addition, the influence exerted by the staining was seen to alter over a period of time. The effect of staining on the naturally occurring lesion which may already contain a number of chromophores and fluorescent substances, however, is uncertain. It may be, therefore, that the observed effects of staining on analysis of artificial lesions are not applicable to natural lesions.

A study was proposed, therefore to investigate the effect, on DIAGNOdent analysis, of the introduction of tea stain into naturally occurring occlusal carious lesions. In addition it was proposed to investigate the potential for influencing the DIAGNOdent analysis by 'removing' stain from a lesion by a bleaching technique.

3.5.2 Materials and Methods

3.5.2.1 Initial Specimen Analysis

50 permanent molar teeth with clinical evidence of occlusal caries were selected from a pool of extracted teeth. The occlusal surfaces were cleaned with a rotating bristle brush and the tooth samples allocated random numbers. Analysis was carried out in order of the random numbers. After drying in a stream of compressed air for 3 seconds, DIAGNOdent analysis of the occlusal surface was performed and the peak value for each tooth was recorded. This analysis was carried out a further 4 times after 3,5,7 and 10 days to establish a baseline peak DIAGNOdent value for the occlusal surface of each tooth. Between each analysis the tooth samples were stored in individual vials containing a cotton pledget moistened with PBS to create a humid buffered environment.

3.5.2.2 Staining and Bleaching of Specimens

A staining solution of tea was prepared by placing one tea bag in 200ml of boiling water and stirring with a magnetic stirrer for 3 minutes. Specimens were randomised and divided into 2 groups of 25. One group was placed into individual vials each containing 5ml of the tea solution and the specimens of the other group were placed into individual vials containing 5ml of 30 vols hydrogen peroxide solution. All samples were stored at 37 degrees centigrade for 24 hours.

3.5.2.3 Analysis of Stained/Bleached Specimens

After 24 hours all samples were removed from their respective vials, dried and DIAGNOdent analysis of the occlusal surface performed immediately. The peak DIAGNOdent value was recorded. This analysis was carried out an additional 4 times after a further 3,4,6 and 7 days. Between each analysis the tooth samples were once again stored in individual vials containing a cotton pledget moistened with PBS to create a humid buffered environment.

Thus a total of 10 analyses of each specimen was undertaken (5 pre-stain and 5 post stain). Table 3.35 summarises the time point in relation to staining of each of these 10 analyses.

Table 3.35. Timing of Specimen Analysis in relation to Staining/Bleaching

Analysis Number	Time Point in relation to Staining/Bleaching
1	9 Days Pre-staining
2	7 Days Pre-staining
3	5 Days Pre-staining
4	3 Days Pre-staining
5	Immediately Pre-staining
6	Immediately Post-staining
7	3 Days Post-staining
8	4 Days Post-staining
9	6 Days Post-staining
10	7 Days Post-staining

3.5.3 Results

3.5.3.1 Repeatability of DIAGNOdent Analysis

The correlations between all possible pre-stain analysis pairings were calculated to demonstrate the stability of the baseline pre-stain reading for each specimen. Results are shown in table 3.36.

Table 3.36. Correlations between Pre-Staining/Bleaching DIAGNOdent Analyses (N=50)

Time Point Pairing	Spearman's Correlation Coefficient	p value
1 vs 2	0.90	0.000
1 vs 3	0.89	0.000
1 vs 4	0.86	0.000
1 vs 5	0.85	0.000
2 vs 3	0.94	0.000
2 vs 4	0.90	0.000
2 vs 5	0.92	0.000
3 vs 4	0.89	0.000
3 vs 5	0.94	0.000
4 vs 5	0.91	0.000

3.5.3.2 Specimens Exposed to Tea Solution

The mean and median DIAGNOdent values of all 25 tea exposed lesions were calculated for each analysis undertaken both before and after staining. The results are shown in table 3.37.

Table 3.37. Mean and Median DIAGNOdent Values at Each Analysis of all Natural Lesions exposed to Tea

Analysis Number	Mean DIAGNOdent Value (S.E.M.)	Median DIAGNOdent Value
1	26.52 (4.14)	19.00
2	28.12 (4.46)	20.00
3	25.12 (4.36)	19.00
4	21.64 (3.85)	16.00
5	25.52 (4.27)	18.00
6	49.84 (4.96)	39.00
7	31.72 (3.87)	30.00
8	32.12 (3.88)	30.00
9	29.64 (4.04)	22.00
10	29.84 (3.99)	27.00

The Wilcoxon Signed Rank test was used to determine the existence of a significant difference between the recorded values immediately before and immediately after exposure to tea. Using this test, a highly significant difference was demonstrated ($p<0.000$).

3.5.3.3 Specimens Exposed to Hydrogen Peroxide

The mean and median DIAGNOdent values of all 25 peroxide exposed lesions were calculated for each analysis undertaken both before and after staining. The results are shown in table 3.38.

Table 3.38. Mean and Median DIAGNOdent Values at Each Analysis of all Natural Lesions exposed to Peroxide.

Analysis Number	Mean DIAGNOdent Value (S.E.M)	Median DIAGNOdent Value
1	31.04 (3.63)	29.00
2	32.08 (3.97)	32.00
3	28.32 (4.08)	25.00
4	27.48 (3.42)	24.00
5	29.04 (3.83)	25.00
6	33.60 (5.55)	26.00
7	25.60 (4.80)	18.00
8	26.40 (5.10)	16.00
9	23.16 (4.46)	15.00
10	24.08 (4.90)	14.00

The Wilcoxon Signed Rank test was used to determine the existence of a significant difference between the recorded values immediately before and immediately after exposure to peroxide. Using this test, no statistically significant difference was demonstrated ($p=0.330$).

3.5.4 Discussion

Each sample was analysed with the DIAGNOdent 5 times prior to either staining or bleaching. This was undertaken in an attempt to demonstrate stability in the DIAGNOdent assessment of the lesions so that any change following the staining would be more apparent as a change from a stable baseline rather than just a single reading. Lussi et al., (1998b) have already demonstrated a high reproducibility for the DIAGNOdent device and data here also indicated highly significant correlations between all 5 analyses. This is also in agreement with the significant intra-examiner correlations demonstrated for DIAGNOdent analysis in sections 2.3.1.1 and 3.3.3.2.

Samples stained by immersion in tea, behaved in a similar way to the artificially created lesions in sections 3.2, 3.3 and 3.4 with a significant increase in DIAGNOdent value following staining. Comparison of the immediate pre-stain with the immediate post-stain DIAGNOdent values indicated a highly significant increase confirming the same staining effect on naturally occurring lesions as was noted on artificially created demineralisation. It is proposed that organic molecules are taken up into spaces within the lesion in the same way and thus exert an influence on the DIAGNOdent analysis. As with the artificial lesions in section 3.4 however, the staining effect was apparently unstable with a reduction in DIAGNOdent value to a new baseline. Plotting of DIAGNOdent value against time for individual samples subjectively demonstrated a similar trend to that of the mean values for all samples (figures 3.6 and 3.7).

Figure 3.6 Plot of DIAGNOdent Value against Time for Specimen No. 22

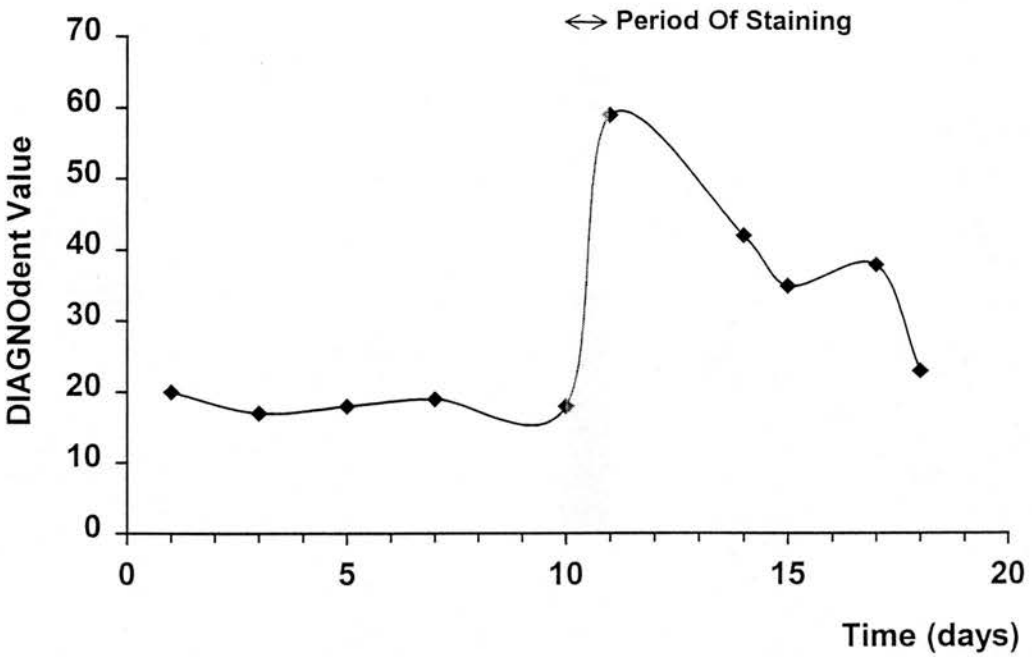
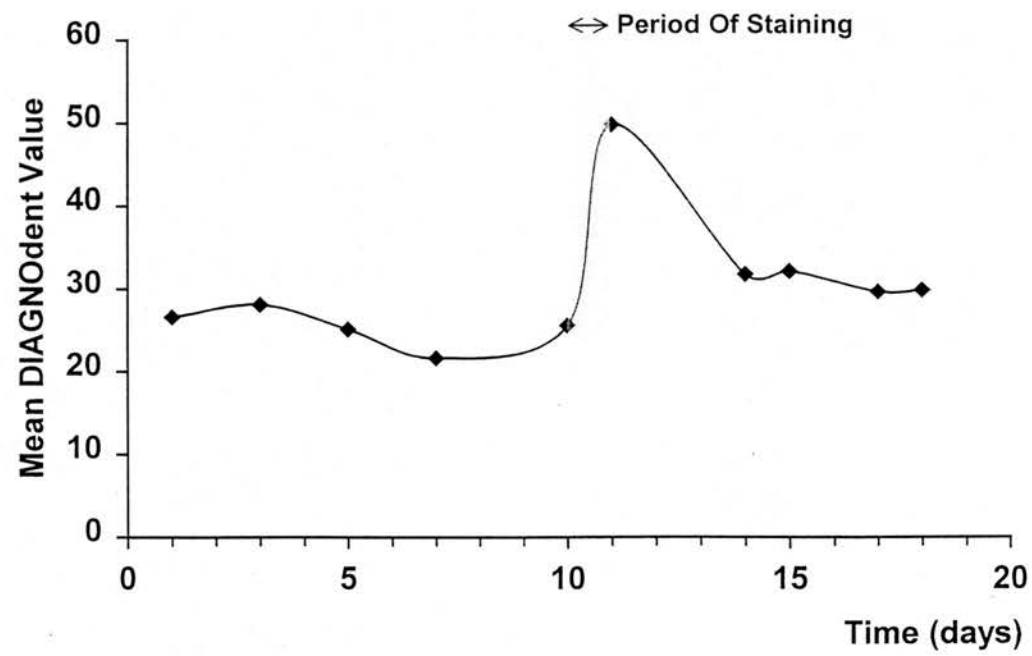


Figure 3.7 Plot of Mean DIAGNOdent Value against Time for all Specimens exposed to Tea Staining



It was concluded, therefore, that this was further evidence for the potential influence of exogenous stain on the DIAGNOdent assessment of caries.

However, the effects of salivary factors may be significant when using *in vitro* models to simulate the caries process as it occurs *in* vivo. Saliva has a complex chemistry and a wide range of biochemical activity that may significantly affect the behaviour of a lesion in the uptake of exogenous stain (Hay 1995). Featherstone et al., (1993) demonstrated the acid resistance *in vitro* of enamel pre-treated with whole saliva. The mineral loss observed in the subsequent creation of artificial lesions was related to the time the enamel was immersed in saliva prior to demineralisation. This would seem to support the theory that the pellicle present on the tooth surface *in vivo* may have a role to play in influencing the exchange of ions between the lesion and its environment. It may also be possible that this influence extends to the movement of organic molecules involved in staining of lesions immersed in tea and it cannot be assumed, therefore, that natural lesions, *in vivo,* would necessarily behave in the same way in response to staining.

Samples exposed to peroxide behaved in a less predictable way. No significant difference between immediate pre-bleaching values and immediate post-bleaching values was evident following analysis of data using the Wilcoxon Signed Rank Test. The potential for a direct influence due to fluorescence of the hydrogen peroxide itself was investigated by carrying out a DIAGNOdent analysis of some hydrogen peroxide solution on a filter paper but this failed to demonstrate any fluorescence and

so it was concluded that introduction of this material into the lesions would not itself impart additional fluorescence. It was hypothesised that the bleaching effect would remove potential fluorophores from the lesion and lower the recorded DIAGNOdent value for the specimen. The specimens were subjectively far less stained following exposure to the peroxide and the presence of less visually detectable staining was clearly evident (figure 3.8).

Figure 3.8 Bleached Tooth Showing Visibly Less Stain

It is possible that immersion of the entire specimen in the bleaching (or staining) solution also exerted an influence on the surrounding sound tooth tissue which was used as a reference point in the DIAGNOdent analysis. Given that the reference point was similarly changed by the bleaching/staining solution, although an actual change had occurred in the lesion, a relative change from its reference may have not be been apparent.

A few individual specimens behaved unusually following exposure to the peroxide with substantial fluctuations in their DIAGNOdent values although it was not possible to account for this in respect of any particular characteristics of these lesions. Although subjective analysis of plotted data for individual lesions indicated some effect on DIAGNOdent values from exposure to peroxide, analysis of data was unable to confirm a predictable change. It was concluded that, in this model, the bleaching did not appear to have the expected effect on DIAGNOdent analysis.

CHAPTER 4

THE DETECTION OF SECONDARY CARIES BY DIAGNOdent ANALYSIS

4.1 Introduction

The majority of all restorations placed in the General Dental Services are replacement restorations and the principle reason for replacement is secondary caries (Mjör 1997). Secondary caries is caries occurring at a tooth restoration interface. It may be residual caries left over following incomplete removal at the initial restoration of the tooth or it may be recurrent caries representing a recurrence of the caries process at a new site. Distinguishing between residual and recurrent caries is of importance since the management of each differs. There is some evidence that residual caries, with a low bacterial load, when sealed beneath a restoration may fail to progress. Recurrent caries, however, is by definition, of a progressive nature and requires intervention (Ricketts et al., 1997).

Clinically, secondary caries is not always straightforward to diagnose and, in certain cases, caries may progress to an advanced stage before becoming clinically evident. In other situations, restorations may be removed due to suspicion of secondary caries which subsequently turn out to be clinically sound. Clinical signs of marginal failure of restorations are not always reliable indicators of the presence of secondary caries (Mjör and Toffenetti 2000) and radiographs can also be difficult to interpret and carry the disadvantage of patient exposure to ionising radiation. A conservative, clinically applicable diagnostic test for secondary caries would be a major advantage for practising dentists and an assessment of the ability of the DIAGNOdent to detect caries at the margin of amalgam restorations was undertaken with this in mind.

4.2 Materials and Methods

4.2.1 Specimen Selection and Preparation

30 permanent molar teeth were selected from a pool of extracted teeth. Teeth were selected on the basis of demonstrating carious lesions extending into dentine. The tooth samples were randomly numbered 1 to 30 and using a dental handpiece and burs, existing restorations and caries was removed from each and cavities prepared for restoration using dental amalgam. Normal clinical criteria of visual appearance and texture of the dental tissues were used to assess the completeness of caries removal. In 20 of the specimens, all enamel margins were cleared of caries and the majority of dentine caries was also removed but in each tooth a small area of carious dentine approximately 2mm by 2mm was left at the amelodentinal junction at a noted point on the cavosurface margin (figure 4.1).

Figure 4.1 Specimen with Residual Caries at Amelodentinal Junction

The point at which caries was left was determined by the location and extent of the original carious lesion. In the remaining 10 specimens, which were designed to act as control specimens, all caries from enamel margins and dentine were removed .

4.2.2 Location of Residual Caries

An indelible mark was placed on the crown of each specimen to indicate the mid point of the buccal surface and this served to ensure easy and consistent orientation of the specimens during subsequent analysis. The position of the residual caries was recorded in terms of the clinically named tooth surfaces as being positioned at one of the following 8 points:

- Buccal
- Distobuccal
- Distal
- Distopalatal / Distolingual
- Palatal / Lingual
- Mesiopalatal / Mesiolingual
- Mesial
- Mesiobuccal

For the test group, the location of the residual caries was recorded in this way for each tooth specimen. DIAGNOdent analysis was carried out to confirm the ability of

the DIAGNOdent device to detect the location of the residual caries at the cavity margin. Each specimen was dried in a stream of compressed air for 3 seconds and the DIAGNOdent device zeroed using sound enamel on the cusp tip. The probe tip of the device was passed over the residual caries varying its position and angulation. The peak value for each specimen was noted.

4.2.3 Restoration of Specimens

All 30 tooth specimens were subsequently restored by normal clinical techniques using a glass polyalkenoate cement base (Chemfil Superior, Dentsply Ltd., Weybridge, England) and amalgam (Amalcap Plus Non Gamma 2, Vivadent, Schaan, Lichtenstein). The 30 restored specimens were placed in individual storage vials containing a cotton pledget moistened with thymol and were held for subsequent analysis.

4.2.4 Analysis of Specimens

Two experienced dentists were invited to examine the restored tooth specimens. Both had some previous experience of using the DIAGNOdent device clinically but were blind as to the location of the residual caries in the specimens. Specimens were placed in random order for analysis and the perceived location of any residual caries at the restoration margins was determined and noted by visual examination under a dental operating light without the aid of magnification. A 2 week period was allowed

to elapse before subsequent DIAGNOdent assessment of the same specimens. Following zeroing on sound enamel at the cusp tip, The DIAGNOdent device, with the 'B' probe tip fitted, was used to examine the restoration margins and the presence and location of any area of caries (as indicated by DIAGNOdent value) at the tooth-restoration interface noted. The locations of residual caries as determined by the visual and DIAGNOdent examinations were compared against each other and against the true locations of residual caries as noted during preparation of the specimens.

4.3 Results

The results of each examiner's assessment of the location of residual caries by both visual and DIAGNOdent assessment along with the actual location noted during sample preparation are presented in appendix 8.

Sensitivities, specificities and positive and negative predictive values for detection of residual caries by visual and DIAGNOdent assessment were calculated for each examiner. Results are shown in table 4.1. The 95% Confidence Intervals for the sensitivity and specificity values were also calculated and are also shown in table 4.1. Details of the statistical method are presented in appendix 11.

Table 4.1. Sensitivities and Specificities for Detection of Residual Caries by DIAGNOdent and Visual Assessment

	Examiner 1		**Examiner 2**	
	Visual	**DIAGNOdent**	**Visual**	**DIAGNOdent**
Sensitivity	0.25	0.44	0.16	0.53
(95% Confidence Interval)	(0.16)	(0.18)	(0.13)	(0.18)
Specificity	1.00	0.71	0.91	0.77
(95% Confidence Interval)	(0.00)	(0.16)	(0.10)	(0.15)
Positive Predictive Value	1.00	0.64	0.75	0.75
Negative Predictive Value	0.40	0.53	0.38	0.56

Statistical analysis of these data was undertaken to determine significant differences between the ability of the visual and DIAGNOdent examinations to detect the residual caries and also to determine any significant differences between the individual examiners. This suggested significant differences between the sensitivities of visual and DIAGNOdent examinations for examiner 2 and the specificities of visual and DIAGNOdent examinations for examiner 1 ($p<0.05$). No other significant differences between the two different examination techniques or examiners were evident. Details of the statistical method are presented in appendix 11.

4.4 Discussion

Presence of ditching or a crevice at the margin of an amalgam restoration is considered by many clinicians as an indication for replacement (Elderton and Nuttall 1983). However, data from research into the relationship between the clinical status of restoration margins and the presence of caries at that margin have failed to demonstrate a correlation between macroscopic features and microscopic caries status (Pimenta et al., 1995). A reliable clinical test for secondary caries represents a major advantage in planning the most appropriate treatment and the validity of the DIAGNOdent in this respect was assessed here.

Considerations in using a fluorescence technique to detect caries adjacent to restorations include the nature of the restorative material. Amalgam has a profoundly different appearance in comparison to enamel and dentine and furthermore does not transmit light. This may have some bearing on the observation of fluorescence at the tooth restoration interface due to light scattering. Hall et al., (1997) have previously investigated the ability of laser fluorescence to detect caries adjacent to amalgam restorations. By comparative studies using TMR, these authors concluded that early artificial lesions adjacent to amalgam restorations can be detected by laser fluorescence and in addition, changes in the mineral content of the lesions can be monitored longitudinally.

In comparison to the above work, the model of secondary caries developed here attempted to simulate the clinical situation more closely. Hall et al., (1997) placed small amalgam restorations into sound buccal surfaces of extracted molar teeth and then, using an artificial lesion creation system, created an area of demineralisation on the surface immediately adjacent to this restoration. The model described here involved the restoration of naturally occurring caries in extracted teeth with the deliberate leaving of an area of carious dentine beneath the margin. Hall et al., (1997) reported sensitivities and specificities of between 0.80 and 0.90 for caries detection in their model.

By comparison, other studies have investigated the ability of visual examination and radiographs to detect secondary caries. Espelid and Tveit (1991) developed an *in vitro* model for assessment of the ability of these techniques to detect secondary caries adjacent to amalgam restorations. Teeth were radiographed and examined visually under 10x magnification and scored for the presence of caries at the restoration margin. Subsequent sectioning and microscopic examination served to determine the true caries status. Radiographic examination alone resulted in correct detection of marginal caries in 29 to 69% of specimens depending on the extent of the caries. When supplemented by clinical diagnosis the rates of correct detection rose to 33 to 89%. In another study, Tveit and Espelid (1986) reported 44% success rate using radiographs in correctly diagnosing caries at restoration margins. The lesions created in this model had margins which did not demonstrate clinically obvious ditching and were therefore comparable to the specimen group resulting in

29% correct radiographic diagnosis in the study of Espelid and Tveit (1991). The positive predictive values of 0.64 and 0.75 demonstrated by the 2 examiners for the DIAGNOdent in detection of secondary caries compared favourably to the findings of the other studies discussed here.

The performance of the DIAGNOdent device in detection of primary caries has been studied more extensively than its ability in the detection of secondary caries at restoration margins. This has been discussed fully in section 1.8 and generally studies by authors such as Lussi et al., (1998a); Lussi et al., (1999a) quote sensitivities for DIAGNOdent analysis of 70-80%. The sensitivities for DIAGNOdent detection of marginal caries demonstrated in this study were of the order of 40-50% and therefore, in common with other diagnostic techniques, it appears that the DIAGNOdent demonstrates superior performance in the detection of primary c.f. secondary caries.

A period of 2 weeks was allowed to elapse between the visual and the DIAGNOdent assessments of the specimens in an attempt to reduce bias in analysing the specimens. Studies by Espelid and Tveit (1991) have shown the improved predictive value of clinical and radiographic examinations together when compared to radiography alone and whilst in the clinical situation any diagnostic test must be considered in the light of clinical judgement, it was felt important to gain an insight into the objective ability of the DIAGNOdent to detect the residual caries rather than assess it as an adjunct to visual examination.

The possibility of fluorescence from the restorative material itself must be considered. However, DIAGNOdent analysis of amalgam in this experiment failed to demonstrate this. In light of the results of sections 3.3 and 3.4 indicating the influence of exogenous stain on DIAGNOdent values, the staining of tooth-restoration interfaces which is seen to occur clinically may also have the potential to cause false positives in the diagnosis of secondary caries. Clinical experience would suggest that this is a potential reality especially when dealing with tooth coloured restorative materials showing signs of marginal failure. However, the opacity of amalgam can make diagnosis of secondary caries more difficult by visual inspection and the use of the DIAGNOdent to penetrate beneath the surface of the tooth-restoration interface is an appealing possibility.

CHAPTER 5

THE DISTRIBUTION OF FLUORESCENCE WITHIN THE CARIOUS LESION

5.1 DIAGNOdent Analysis of the Pattern of Fluorescence within Natural Carious Lesions: I Transverse Dissection

5.1.1 Introduction

The exact nature of the fluorophores in naturally occurring carious lesions is not completely understood. Studies by Hafström-Björkman et al., (1991) analysing the emission spectra of enamel fractions have suggested both organic and inorganic components were involved in fluorescence. More recently studies by Hibst and Paulus (1999) and Hibst and Paulus (2000) have used a DIAGNOdent device to investigate fluorescence from various oral tissues and also from bacteria. It was proposed by these authors that the fluorescence measured by the DIAGNOdent may result from the absorption of organic molecules onto the teeth. The increased fluorescence seen in carious lesions is suggested to result from bacterial metabolites rather than as a direct result of the demineralisation of the tooth substance. However, beyond these limited analyses, little is known about the nature or distribution of the fluorescent molecules within a carious lesion.

This experiment was designed to allow some investigation into the distribution of the fluorophores within a carious lesion. It is not known whether the recorded fluorescent signal is related to surface absorption of stain onto the lesion or whether the proposed fluorophores of bacterial origin would be found distributed throughout the lesion or only in discrete zones.

5.1.2 Materials and Methods

5.1.2.1 Specimen Selection and Visual Assessment

Twenty extracted molar teeth exhibiting varying degrees of occlusal caries were selected for analysis. Selection was made on the basis of clinical evidence of caries in the absence of gross cavitation. For each tooth, the occlusal surface was cleaned using a rotating bristle brush and a site on the occlusal surface chosen for investigation. The site chosen in each case was an interlobal groove where clinical evidence of carious attack was seen. The visual appearance of the carious lesion at this point was recorded and classified according to the criteria of Ekstrand et al., (1998) (Table 5.1). This was taken to represent the clinical caries score for the lesion.

Table 5.1. Criteria for Clinical Classification of Occlusal Caries

Score	Criteria
0	No or slight change in enamel translucency after air drying for 5 seconds
1	White opacity hardly visible on the wet surface but distinctly visible after air drying
1a	Brown opacity hardly visible on the wet surface but distinctly visible after air drying
2	White opacity distinctly visible without air drying
2a	Brown opacity distinctly visible without air drying
3	Localised enamel breakdown in opaque or discoloured enamel and/or greyish discolouration from the underlying dentine
4	Cavitation in opaque or discoloured enamel exposing the dentine beneath

5.1.2.2 Specimen Preparation

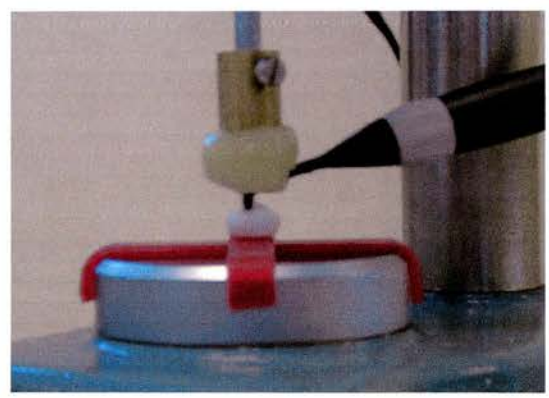
The tooth specimen was decoronated at the cement-enamel junction using a diamond cutting disc (Labcut 1010, Agar Scientific Ltd, D.R. Bennett Ltd, Leceister, England) and the coronal portion fixed, with cyano-acrylate adhesive, onto a custom made acrylic resin mounting jig (figure 5.1). The acrylic mounting jig was designed to fit precisely onto the measuring table of a micrometer (Mitutoyo Digimatic Indicator, Mitutoyo MFG Co. Ltd, Japan) (figures 5.2 & 5.3) and to allow serial removal and replacement of the specimen with consistency of positioning. Accurate repositioning of the mounted specimen was effected by aligning the edge of the acrylic mounting block with a scribed mark on the measuring table of the micrometer. The DIAGNOdent 'A' tip was rigidly fixed to the moveable measuring arm of the micrometer. The DIAGNOdent probe was attached by acrylic resin to a custom made brass block which fitted precisely onto the measuring tip of the micrometer instrument (figure 5.4). This allowed straightforward attachment and removal of the DIAGNOdent probe from the micrometer when required.

Figure 5.1 Specimen Mounted on Acrylic Mounting Block

Figure. 5.2 Mitutoyo Digimatic Indicator

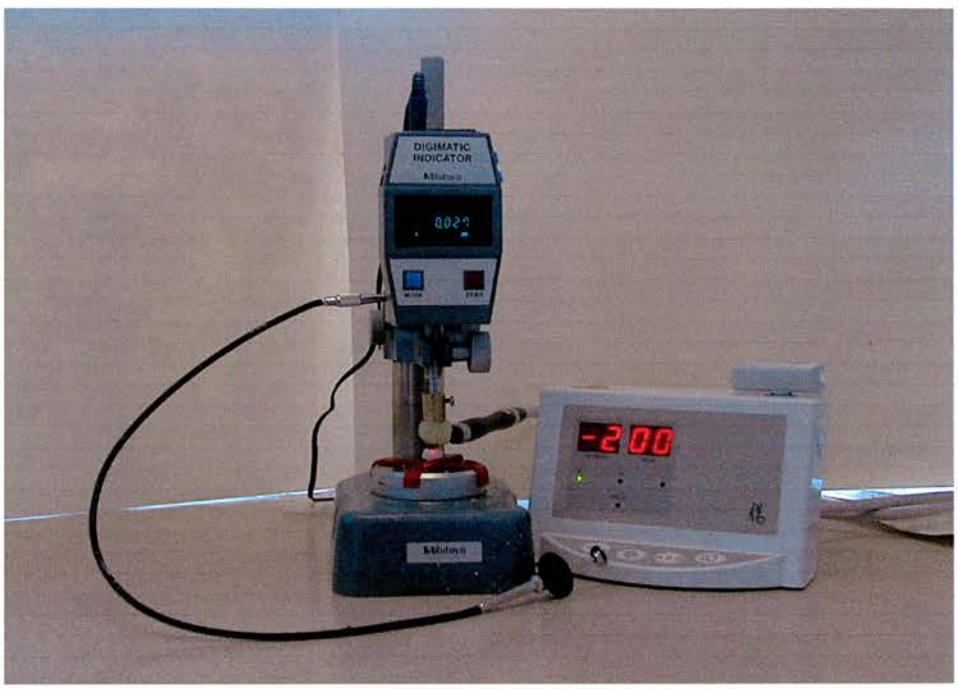
Fig. 5.3 Acrylic Mounting Jig Fitting onto Table of Micrometer

Fig. 5.4 Attachment of DIAGNOdent Tip to Measuring Tip of Micrometer



The mounting of the tooth specimen and DIAGNOdent probe tip on a jig in this way created a reproducible and consistent relationship during subsequent measurements. In addition, micrometer and DIAGNOdent readings could be obtained simultaneously for each probe tip position (figure 5.5).

Figure 5.5 Simultaneous Recording of Micrometer and DIAGNOdent Readings



5.1.2.3 DIAGNOdent Analysis

The DIAGNOdent was given a baseline zero value of sound enamel on the cusp tip in the usual way and an initial DIAGNOdent value for the carious lesion at the chosen site was recorded. The micrometer was also set to zero at this point indicating the level of the base of the fissure as measured by the dimensions of the measuring probe tip of the DIAGNOdent. The tooth specimen on its acrylic mounting block was then removed and the occlusal surface ground to remove the cusps to the level of the base of the fissure at the chosen site. This level was confirmed by an unchanged reading on the micrometer on replacement of the specimen on the measuring table of the micrometer. Grinding was carried out using a rotating abrasive stone wheel and fine reduction carried out by hand lapping on a glass slab with a slurry of 50 micron aluminium oxide powder (White Bauxlite 2000 Honing Abrasive, Raymond A. Lamb, London) (figure 5.6).

Figure 5.6 Hand Lapping Apparatus

The tooth sample was washed in a compressed air/water spray for three seconds and dried for three seconds in compressed air. The sample was then remounted on the micrometer and the DIAGNOdent tip, attached to the measuring arm of the micrometer, was lowered onto the tooth surface. A DIAGNOdent value was recorded at this point. The specimen, on its acrylic mounting block, was removed once again and a further 100μm ground from the occlusal surface. Following washing and drying, the specimen was replaced on the micrometer and the amount of material removed from the tooth surface confirmed before recording a new DIAGNOdent value at this level. This procedure was repeated, removing 100μm increments from the surface of the carious lesion, until the DIAGNOdent readings fell below the threshold value of 9 indicating that the full extent of the lesion had been examined and the probe tip was contacting sound tooth tissue. When the caries was more extensive and the DIAGNOdent value remained above 9, sequential 100μm increments were removed until the pulp chamber was reached.

5.1.3 Results

5.1.3.1 Distribution of Fluorescence

The DIAGNOdent value at each point within the lesion was plotted against the distance into the lesion as measured from the base of the fissure. Plotted data for each specimen are shown in figures 5.7 -5.26

Figure 5.7 Plotted DIAGNOdent Values within Lesion (Specimen 1)

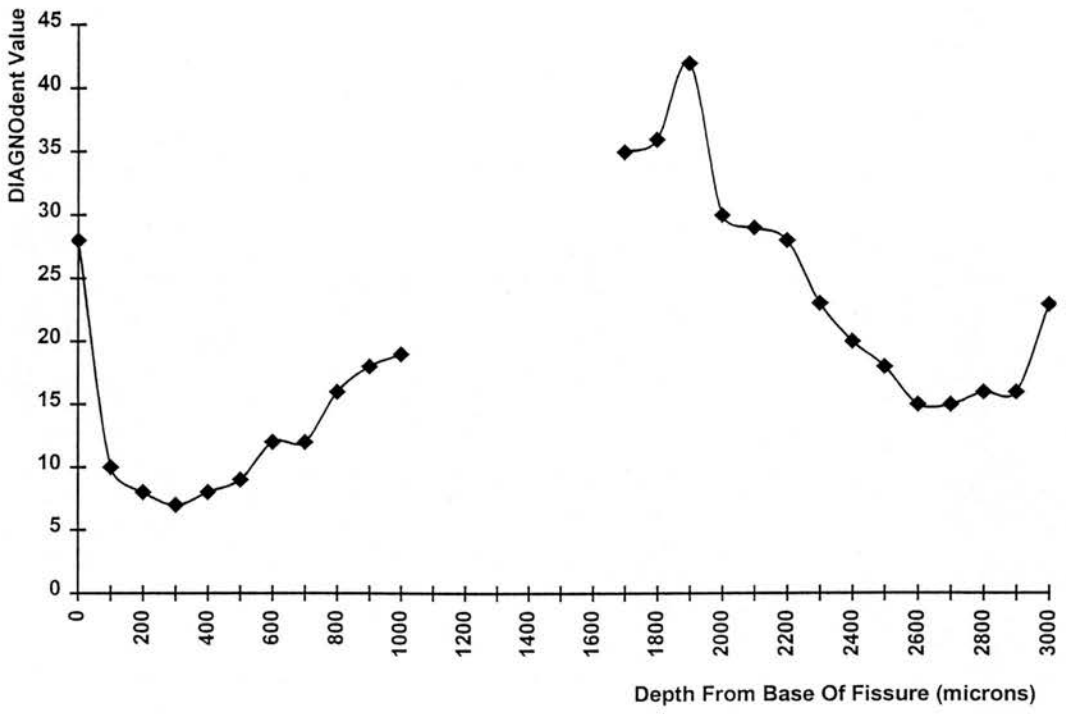


Figure 5.8 Plotted DIAGNOdent Values within Lesion (Specimen 2)

Figure 5.9 Plotted DIAGNOdent Values within Lesion (Specimen 3)

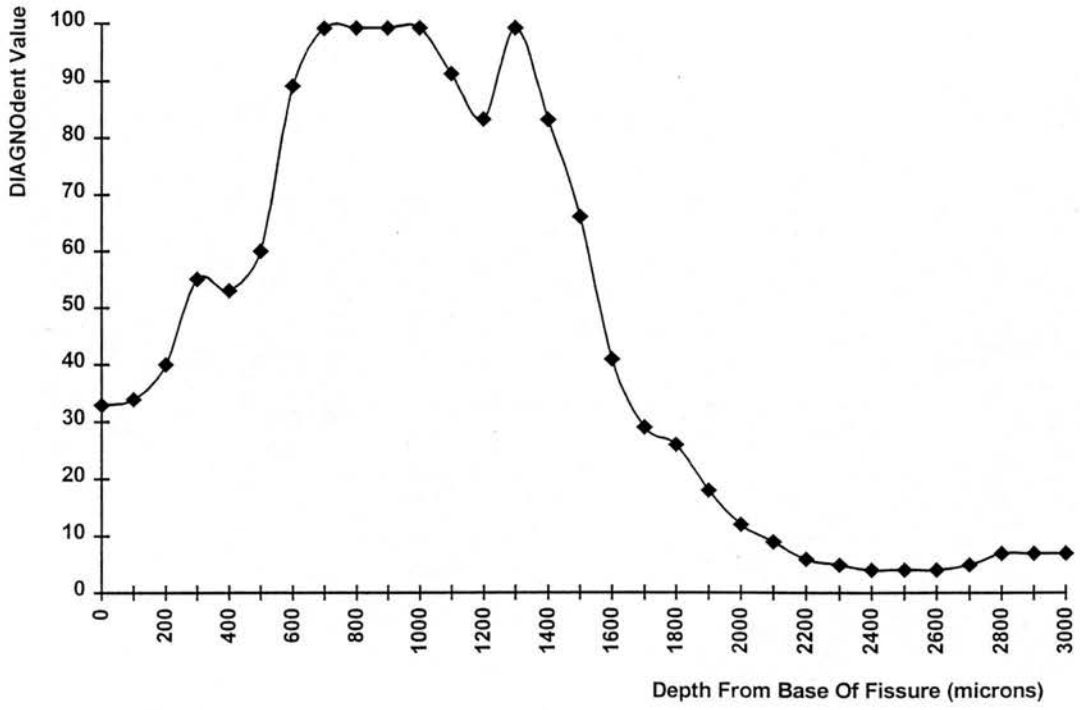


Figure 5.10 Plotted DIAGNOdent Values within Lesion (Specimen 4)

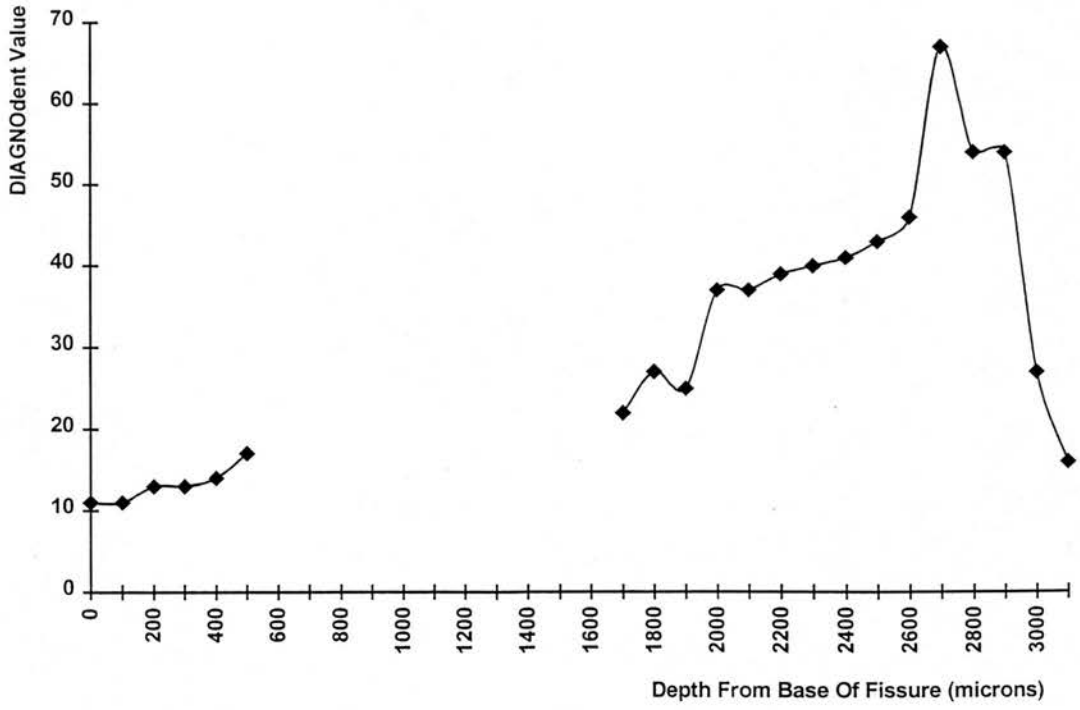


Figure 5.11 Plotted DIAGNOdent Values within Lesion (Specimen 5)

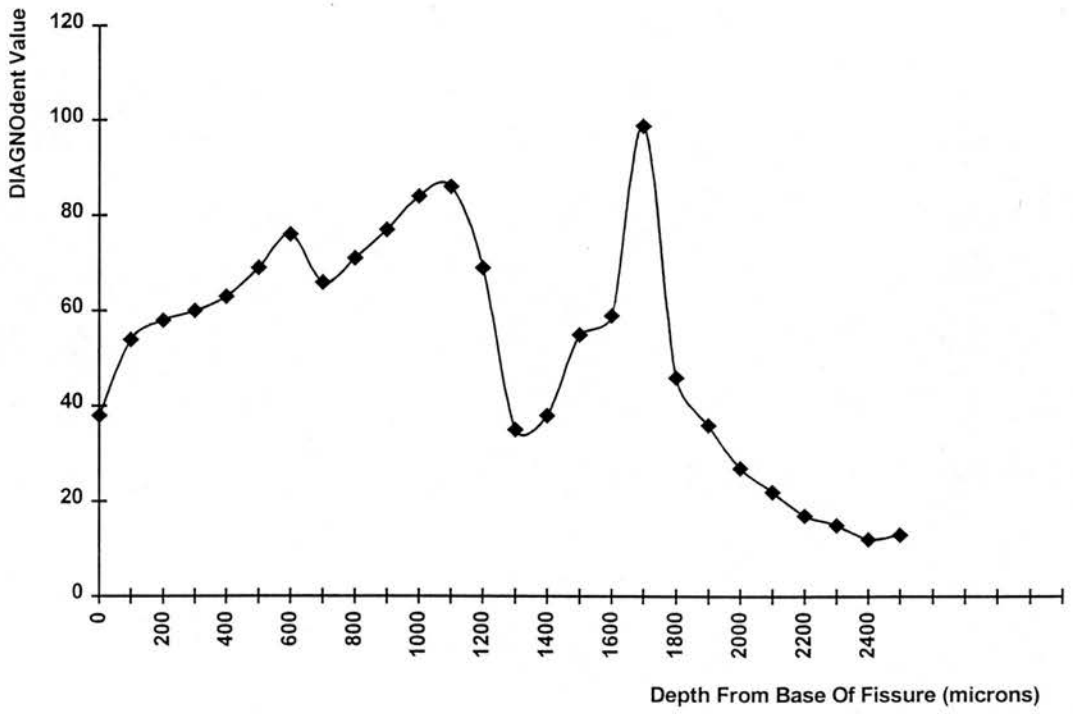


Figure 5.12 Plotted DIAGNOdent Values within Lesion (Specimen 6)

Figure 5.13 Plotted DIAGNOdent Values within Lesion (Specimen 7)

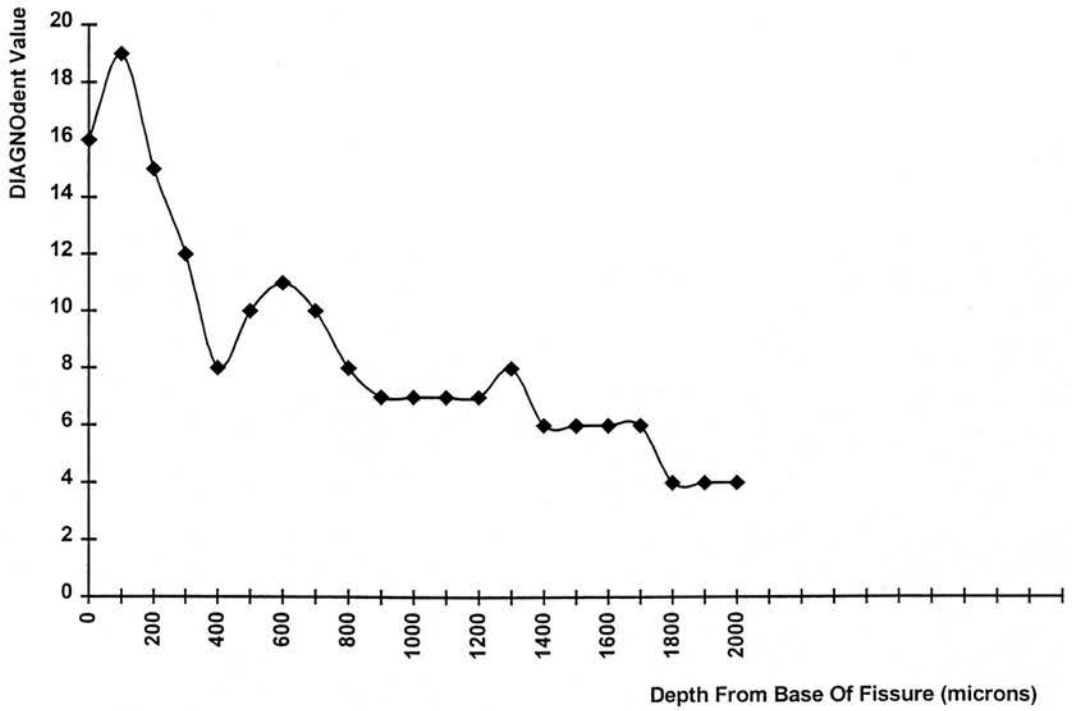


Figure 5.14 Plotted DIAGNOdent Values within Lesion (Specimen 8)

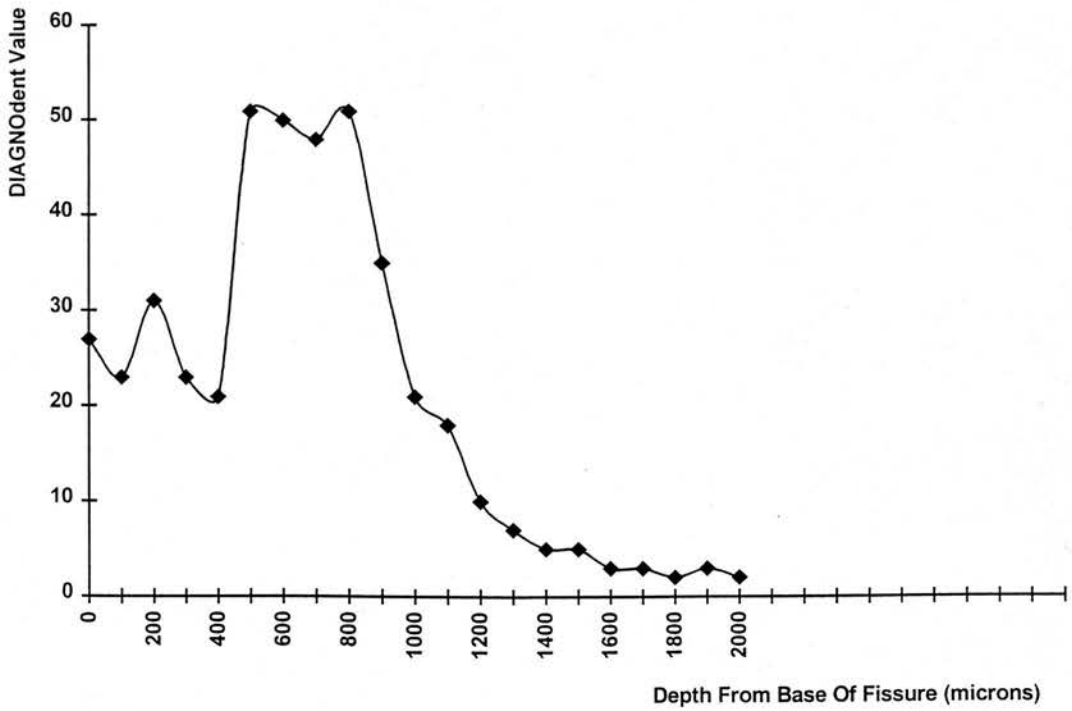


Figure 5.15 Plotted DIAGNOdent Values within Lesion (Specimen 9)

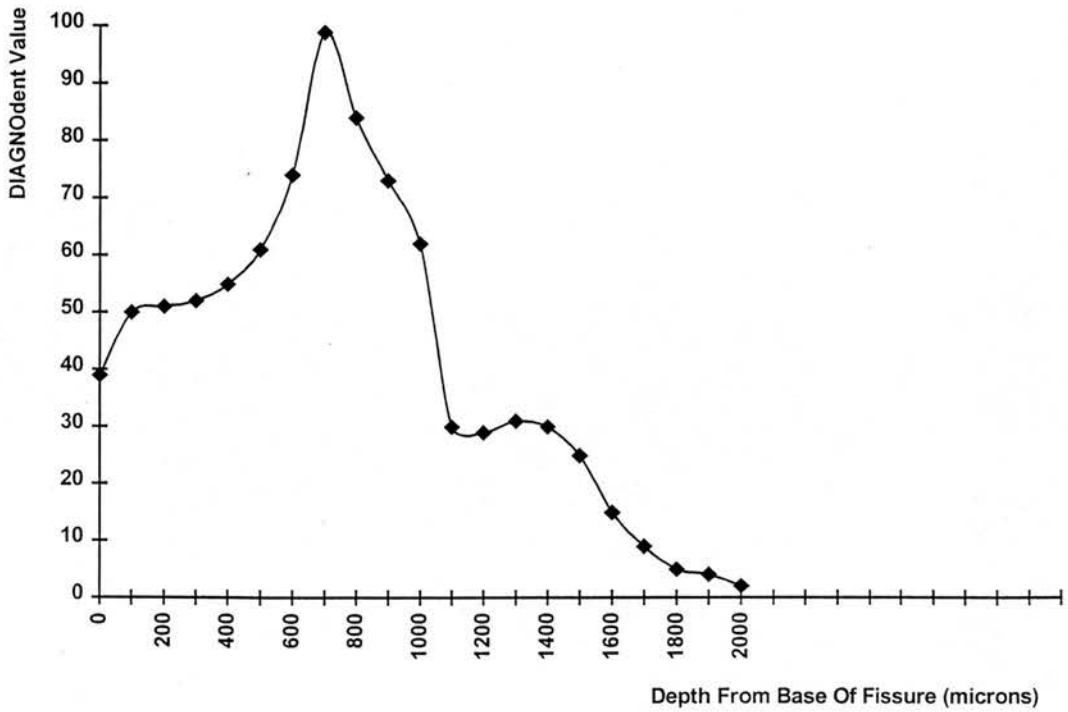


Figure 5.16 Plotted DIAGNOdent Values within Lesion (Specimen 10)

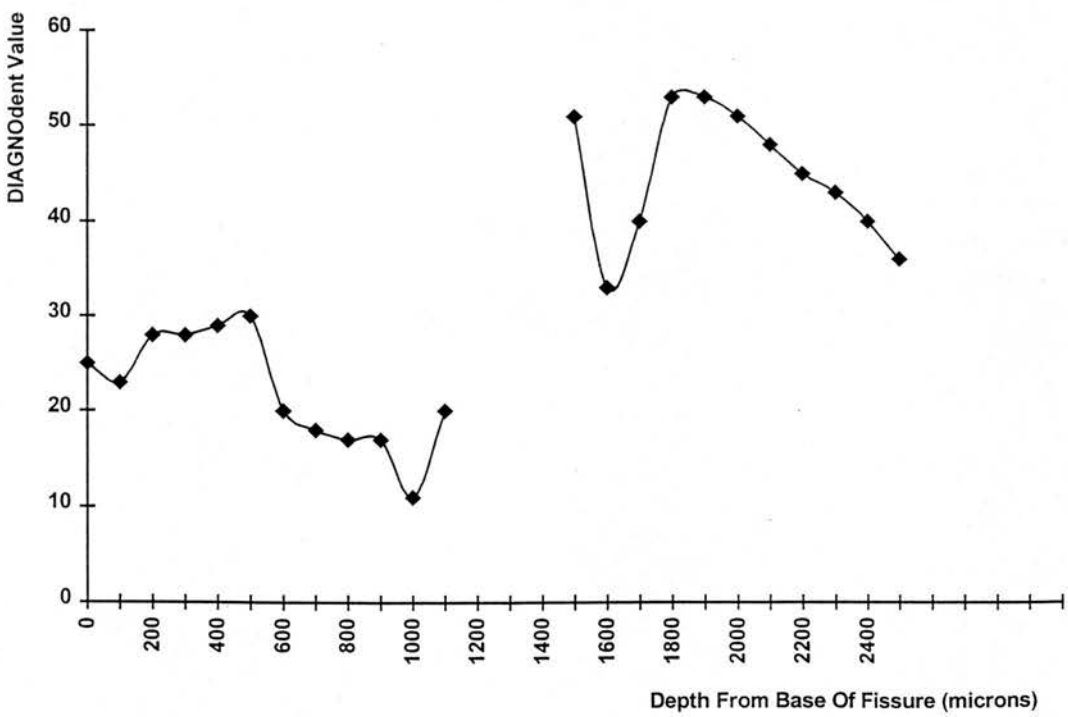


Figure 5.17 Plotted DIAGNOdent Values within Lesion (Specimen 11)

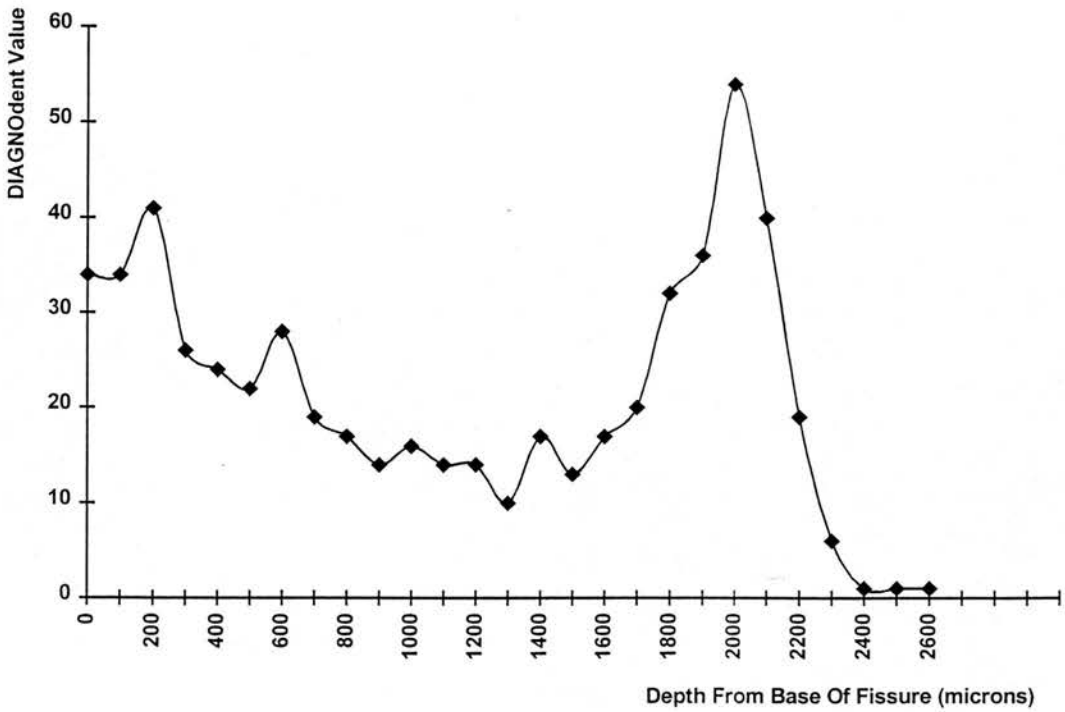


Figure 5.18 Plotted DIAGNOdent Values within Lesion (Specimen 12)

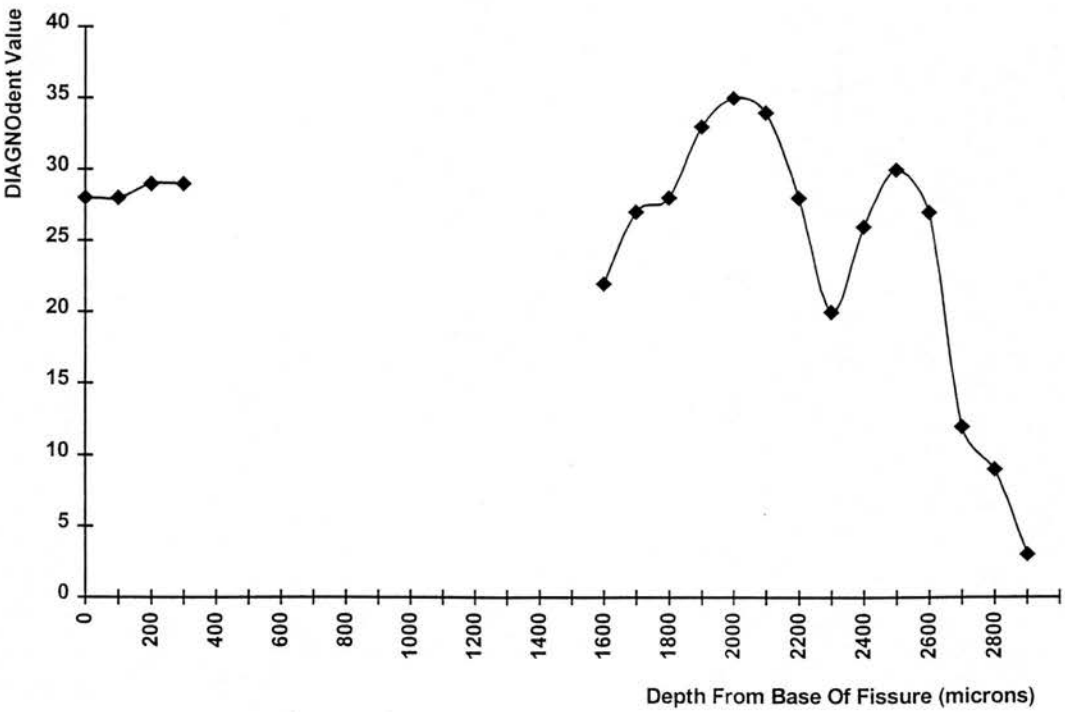


Figure 5.19 Plotted DIAGNOdent Values within Lesion (Specimen 13)

Figure 5.20 Plotted DIAGNOdent Values within Lesion (Specimen 14)

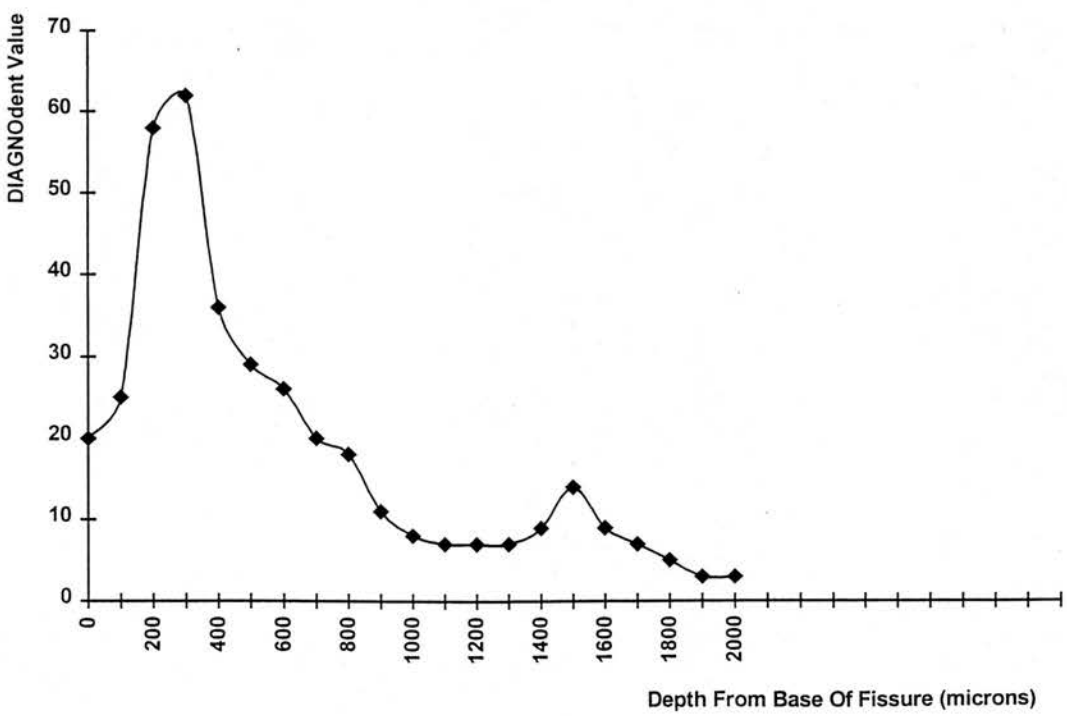


Figure 5.21 Plotted DIAGNOdent Values within Lesion (Specimen 15)

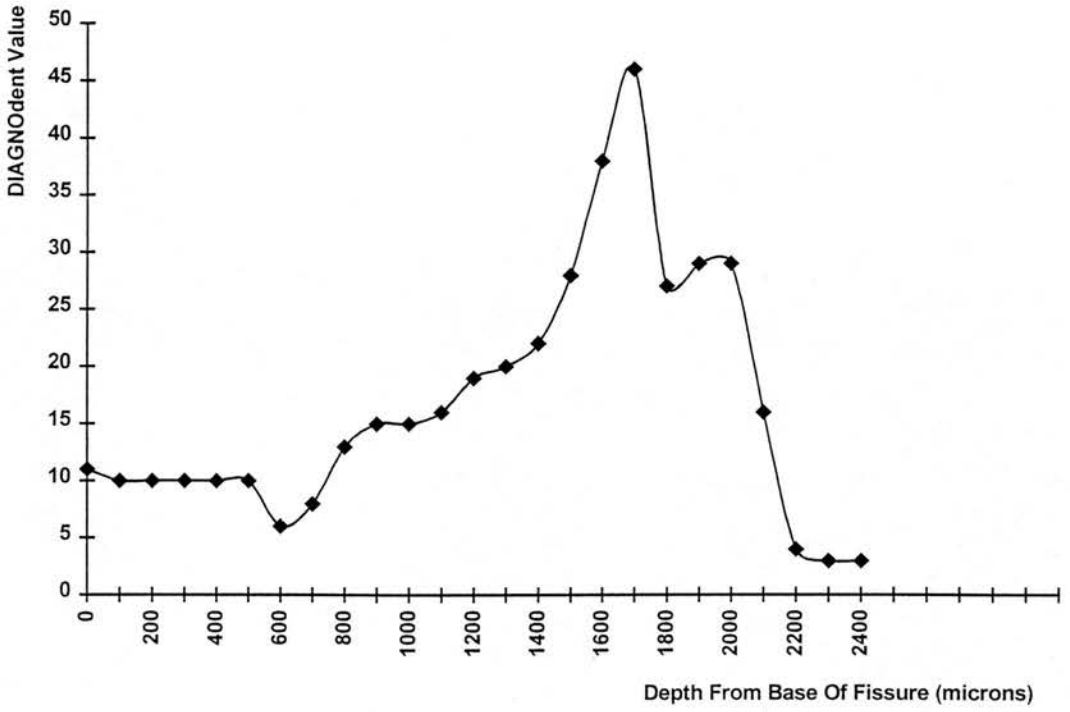


Figure 5.22 Plotted DIAGNOdent Values within Lesion (Specimen 16)

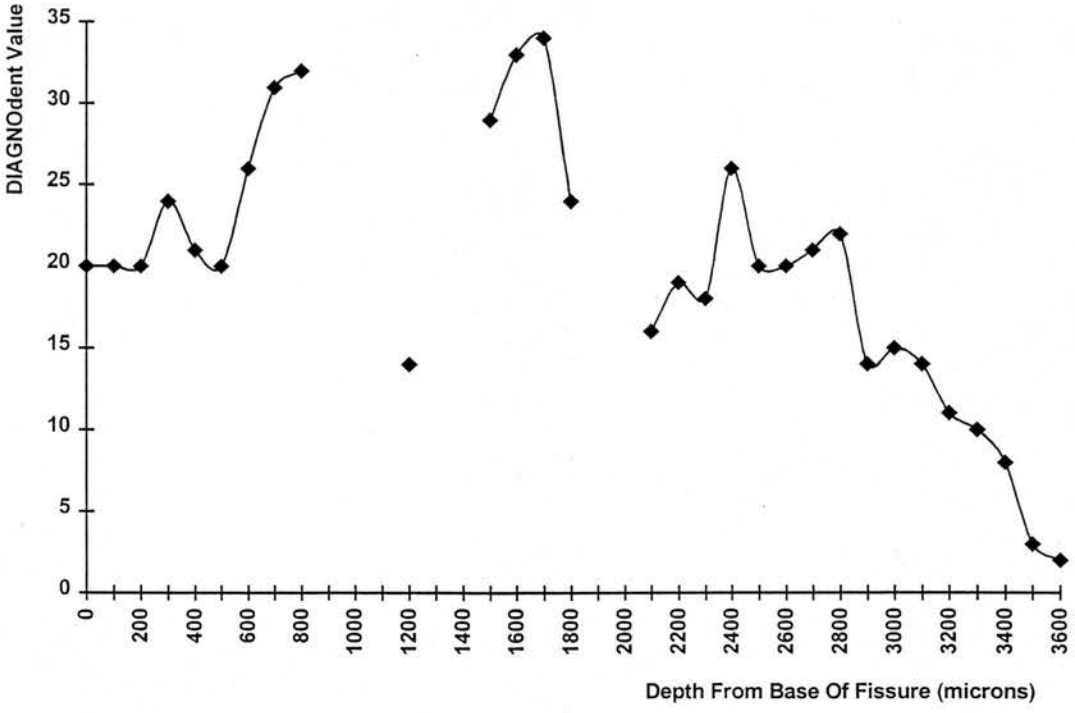


Figure 5.23 Plotted DIAGNOdent Values within Lesion (Specimen 17)

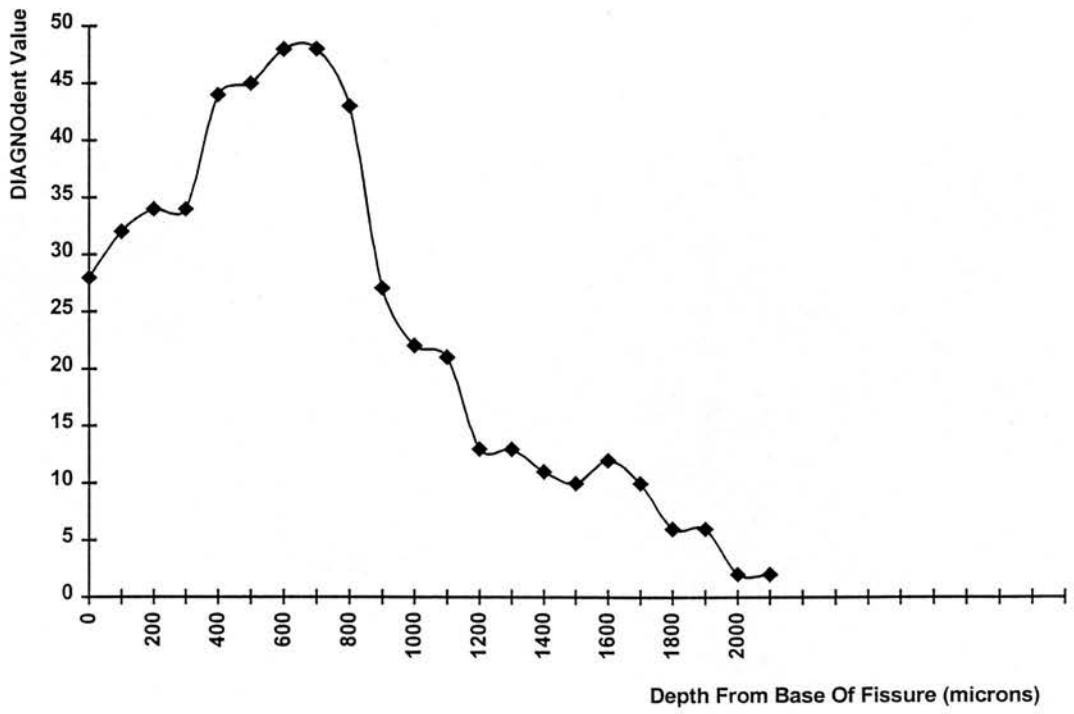


Figure 5.24 Plotted DIAGNOdent Values within Lesion (Specimen 18)

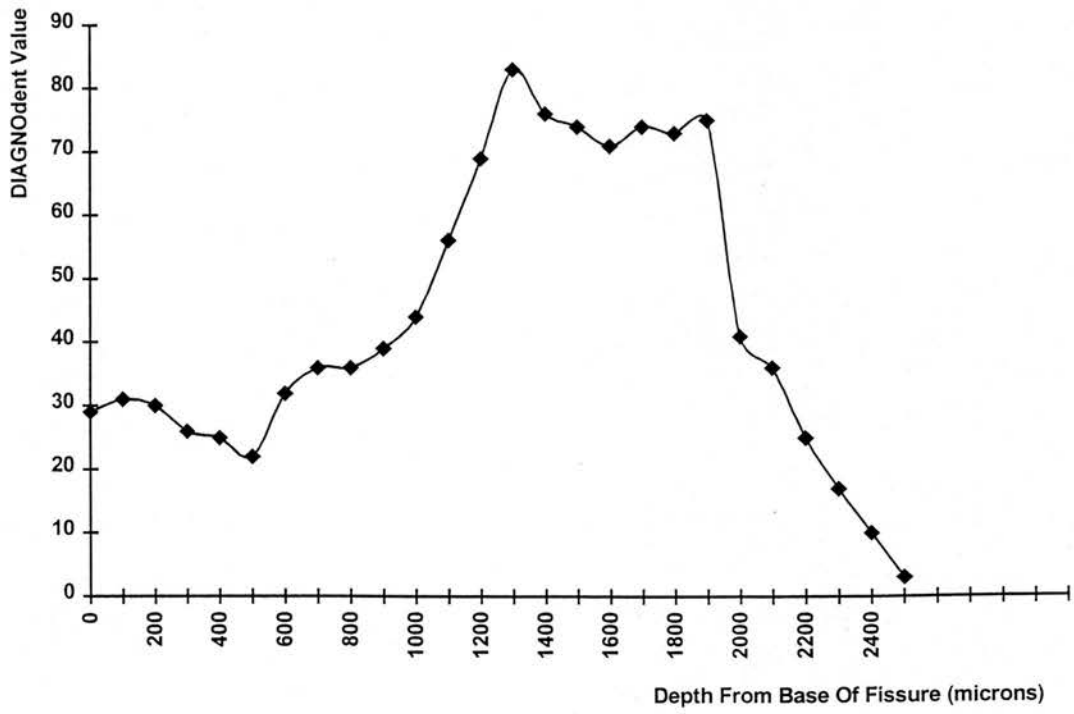
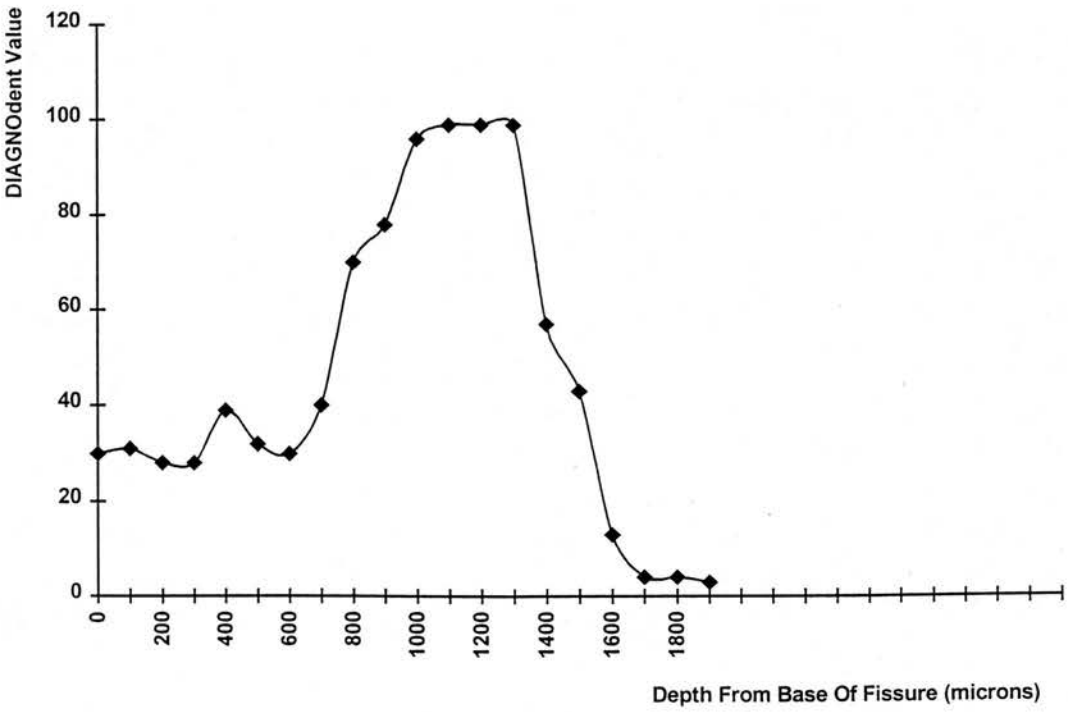


Figure 5.25 Plotted DIAGNOdent Values within Lesion (Specimen 19)

Figure 5.26 Plotted DIAGNOdent Values within Lesion (Specimen 20)



5.1.3.2 Correlation between Visual and DIAGNOdent Assessment of Lesions

The initial DIAGNOdent value for each intact lesion, prior to any alteration by grinding of the occlusal surface, was recorded. In addition, a mean DIAGNOdent value for each specimen was determined by calculating the mean of all readings obtained during subsequent lesion dissection. From the plots of DIAGNOdent value against depth from the base of the fissure (DDV plot), the peak value for each specimen, recorded during the stepwise analysis, was noted along with the depth at which the DIAGNOdent value fell below the threshold level of 9. Furthermore, the area under the DDV plot to this point (or to the point of pulp exposure) was calculated for each individual lesion. The above data are shown for each specimen in tables 5.2 and 5.3. These data were studied for correlation between the recorded parameters and correlation co-efficients are shown in table 5.4.

Table 5.2. Summary Data for DIAGNOdent Analysis of Transverse Dissection of Lesions (Specimens 1-10)

Specimen No.	Clinical Caries Score	Initial DIAGNOdent Value	Area under DDV Curve	Mean DIAGNOdent Value	Peak DIAGNOdent Value	Lesion Depth (μ)
1	3	72	106150	35.06	42	3000
2	2a	21	52950	28.68	48	1800
3	2a	33	129600	57.52	99	2100
4	3	10	172950	54.47	54	3100
5	2a	30	131950	51.73	99	2500
6	1a	11	29400	14.47	21	2000
7	2a	12	9700	12	19	800
8	1a	26	39900	29.71	51	1300
9	3	50	85200	46	99	1800
10	3	27	102550	40.62	53	2500

Table 5.3. Summary Data for DIAGNOdent Analysis of Transverse Dissection of Lesions (Specimens 11-20)

Specimen No.	Clinical Caries Score	Initial DIAGNOdent Value	Area under DDV Curve	Mean DIAGNOdent Value	Peak DIAGNOdent Value	Lesion Depth (μ)
11	3	27	54700	23.63	54	2300
12	2	28	162050	54.53	35	2900
13	1a	22	153350	51.7	99	2900
14	1	16	35950	20.72	62	1700
15	1	20	40450	17.91	46	2200
16	3	17	103300	30.56	34	3400
17	1a	22	48400	26.37	48	1800
18	1a	20	111700	43.58	83	2500
19	3	21	93700	39.79	99	2300
20	3	17	89900	50.89	99	1700

Table 5.4. Correlations between Recorded Parameters in Transverse Lesion Dissection

Parameters	Spearman's Rank Correlation Coefficient
Clinical Caries Score vs Area under DDV Curve	0.34
Clinical Caries Score vs Mean DIAGNOdent Value	0.32
Clinical Caries Score vs Initial DIAGNOdent Value	0.23
Clinical Caries Score vs Lesion Depth	0.32
Clinical Caries Score vs Peak DIAGNOdent Value	0.17
Initial DIAGNOdent Value vs Area under DDV Curve	0.35
Initial DIAGNOdent Value vs Mean DIAGNOdent Value	0.42
Initial DIAGNOdent Value vs Lesion Depth	0.17
Initial DIAGNOdent Value vs Peak DIAGNOdent value	0.29
Peak DIAGNOdent Value vs Mean DIAGNOdent Value	0.59*
Peak DIAGNOdent Value vs Lesion Depth	-0.02
Peak DIAGNOdent Value vs Area under DDV curve	0.39
Mean DIAGNOdent Value vs Lesion Depth	0.48*
Mean DIAGNOdent Value vs Area under DDV Curve	0.89*

* denotes statistical significance at $p<0.05$ level.

5.1.4 Discussion

5.1.4.1 Practical Considerations

Devising an experimental method for studying the variation in DIAGNOdent value within a carious lesion presented considerable practical difficulty. It was desirable to measure the DIAGNOdent value at fine increments within the lesion and direct measurement on the lesion surface therefore required sequential removal of precise amounts of tissue from the lesion surface. Using available sectioning apparatus (Microslice 2 Precision Slicing Machine, Malvern Scientific Instruments, Malvern, England), (figure 5.27) stepwise removal of 700μm increments was achievable but this was considered inadequate for the purposes of this study. Sequential grinding and checking of specimen thickness was therefore the method employed to remove 100μm increments from the lesion surface. This was a labourious technique but was considered necessary to enable the required detailed analysis of the specimens.

Figure 5.27 Microslice 2 Precision Slicing Machine

Reproducible specimen positioning after removal of each increment was also difficult to achieve. Therefore the tooth specimen was firmly attached, using cyanoacrylate resin, to a custom made acrylic mounting jig which could be removed and replaced, with consistency of position, onto the micrometer table.

The starting point for analysis was taken to be the base of the fissure. This point was difficult to locate in practice and to allow some clinical validity of the technique, the point chosen was the maximum penetration of the fissure system achievable with the DIAGNOdent probe tip. In clinical use, this is the point of the fissure which is examined. The DIAGNOdent value at this point was accepted as the initial assessment of each lesion under investigation.

The first grinding of the sample was designed to remove the occlusal surface down to the base of the fissure as determined by the position of the DIAGNOdent probe tip and in practice this point was determined by grinding and lapping away the surface as far as the reading on the micrometer remained unchanged thus indicating that the probe tip was still at the same point on the lesion surface.

In practice this removal of the occlusal surface and adjacent cusp slopes was seen to have an effect on the DIAGNOdent value of that point of the lesion surface. In the majority of specimens (14/20) the initial removal of the rest of the occlusal surface had the effect of increasing the DIAGNOdent value. This may have been due to allowing a deeper penetration of the DIAGNOdent probe into the lesion after

removing the obstruction of adjacent cusp slopes. However, if accurate maintenance of the probe position was maintained, this should not have been the case. Other specimens showed a decrease in DIAGNOdent value following the initial removal of the adjacent cusp slopes and this may indicate the contribution of fluorescence from around all surfaces of the probe rather than simply at its tip. This proposal is supported by evidence from Juhl (1983) who analysed the location of caries within the fissure system of teeth. He observed that caries was located in the top and bottom part of the wall of the fissure as well as below its base. Most frequently the carious lesion was localized in the lower part of fissures but in may cases multiple foci of caries were found in both the upper and lower parts.

However, only a small proportion of teeth examined by Juhl (1983) had carious lesions positioned above the entrance to the fissure system and this would support the observation here of an increase in DIAGNOdent value as the probe tip was allowed to penetrate deeper into the fissure system. It may be, therefore, that in this particular situation of analysis within the fissure, that the DIAGNOdent value was a reflection of the tooth status in an area all around the end of the probe rather than restricted to its very tip. This raises the question of the spatial resolution of the instrument and no data is available from the manufacturers to indicate the limitations of the device in this respect.

The possibility of debris from the grinding process influencing the DIAGNOdent assessment of the specimens was considered. However, DIAGNOdent examination

of the grinding stone, and the aluminium oxide powder used, failed to demonstrate fluorescence from either of these materials. Furthermore, a strict washing protocol was followed to ensure thorough removal of all debris from the specimen before each analysis.

5.1.4.2 Pattern of Fluorescence

The variation in DIAGNOdent value as further sequential 100μm increments were removed did not appear to follow a consistent pattern. All specimens exhibited a peak DIAGNOdent value at some point within the lesion but the position of that peak within the lesion appeared variable. Some specimens e.g. Nos. 7, 14, 17 demonstrated an early peak in the superficial layers of the lesion followed by a gradual decline in DIAGNOdent value whereas others showed a gradual increase in DIAGNOdent value towards a peak in the deeper part of the lesion e.g. Nos. 4, 13, 15, 18. A number of specimens showed decreasing values after a peak followed by another rise to a second peak at a deeper level e.g. Nos. 2, 5.

DIAGNOdent value was plotted against vertical position within each lesion relative to the base of the fissure under study and the area under this 'depth-DIAGNOdent value' (DDV) curve was used in this analysis as an estimate of the 'total caries content' of the lesion as assessed by DIAGNOdent analysis. This is not a previously recognised parameter but was considered here as it reflects the degree of demineralisation as measured by the lesion depth *and* the magnitude of the

DIAGNOdent values within the lesion. This was intended to allow comparison of shallow lesions with high DIAGNOdent values with deeper lesions with lower DIAGNOdent values. The value 'Area under DDV curve' was seen to correlate significantly with the mean DIAGNOdent value and may thus represent a valid means of expressing the 'total fluorescence' within a lesion.

Some practical problems in assessing the DIAGNOdent value at the chosen 100μm interval were presented when areas of cavitation were encountered in certain lesions. Areas of cavitation caused the DIAGNOdent probe to drop more than a further 100μm into the lesion and no reading was possible at the cavitated point. Consequently, the true DIAGNOdent value for this portion of the lesion was unknown. This created a problem in subsequent data analysis and it was unclear as to the most appropriate way of accounting for this missing data. In order to examine the spread of fluorescence and to calculate the mean DIAGNOdent value and area under the DDV curve for the whole lesion, it was necessary to assume a value for the depths at which cavitation had occurred. Given the significant tissue destruction at this point it was considered reasonable to assume a maximum DIAGNOdent value of 99 for all cavitated points and therefore this was the value chosen. Consideration was also given to omitting this portion of the lesion in analysis but this was deemed inappropriate as it meant effectively ignoring the presence of a large area of extensively demineralised tissue.

The lesion depth was determined by noting the point, relative to the starting point of the base of the fissure, at which the DIAGNOdent value fell below the threshold value of 9 for clinically significant caries.

5.1.4.3 Correlation of DIAGNOdent Analysis with Other Parameters

Data were analysed using Spearman's Correlation Coefficient for correlations between the initial DIAGNOdent value and the parameters of clinical caries score; area under the DDV curve; mean DIAGNOdent value; peak DIAGNOdent value and lesion depth. No significant correlations between these data were evident. Similarly correlations between the clinical caries score and the parameters of area under the DDV curve; mean DIAGNOdent value; peak DIAGNOdent value and lesion depth were found to be non-significant. However, a significant correlation was found between the mean DIAGNOdent value and the lesion depth. In addition, mean DIAGNOdent value during lesion dissection correlated significantly with the peak DIAGNOdent value and the area under the DDV curve for each lesion.

Although specificity and sensitivity values for DIAGNOdent assessment of caries have been reported as comparable to other techniques of diagnosis when compared to a gold standard (Longbottom et al., (1999a); Côrtes and Ellwood (2000)), direct comparison between DIAGNOdent assessment of carious lesions and other methods (including visual inspection) have been reported elsewhere with varying results. Using a variety of techniques, other workers have failed to report clear

relationships between DIAGNOdent value and other parameters. Sheehy et al., (2000) demonstrated a significant correlation between a visual ranking scale and DIAGNOdent value in occlusal caries in molar teeth. However, they also noted that lesions appearing similar on the clinical scoring system had markedly different DIAGNOdent values indicating a problem in drawing direct comparison between these two methods of lesions assessment.

Furthermore Iijima and Takagi (2000) in TMR analysis of sections from artificial lesions which had undergone a de/remineralisation cycle, stated that they were unable to demonstrate a simple linear relationship between DIAGNOdent value and either mineral loss or lesion depth.

The experimental method employed in this study also failed to demonstrate any strong correlation between the DIAGNOdent value at the lesion surface and the extent of the caries as determined by DIAGNOdent assessment during subsequent lesion dissection. Whilst it is recognised that the particular experimental methodology may not have allowed a true and accurate assessment of the lesion, it may be that DIAGNOdent assessment of occlusal caries is not a good indicator of true caries extent. In addition, subjective analysis of plotted data revealed no discernible pattern of fluorescence distribution as recorded by the DIAGNOdent within the lesion.

5.2 DIAGNOdent Analysis of the Pattern of Fluorescence within Natural Carious Lesions: II Longitudinal Dissection

5.2.1 Introduction

The experimental method described in section 5.1 has suggested no obvious pattern of fluorescence within occlusal caries. As a result, it was decided to attempt development of an alternative methodology to study the relationship of the DIAGNOdent assessment of a lesion with its extent and the distribution of fluorescence therein.

5.2.2 Materials and Methods

5.2.2.1 Specimen Selection and Visual Assessment

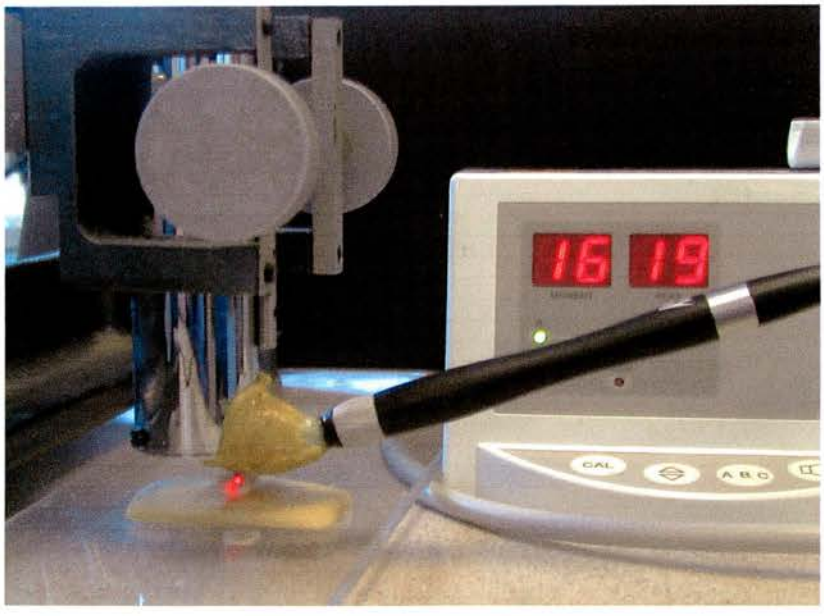
Twenty four molar teeth were selected from a pool of extracted teeth. Selection was made on the basis of visual evidence of non-cavitated occlusal caries. Selected tooth specimens were cleaned using a rotating bristle brush and oil free prophylactic paste. Carious lesions were dried and examined under a dental operating light and scored according to the criteria of Ekstrand et al., (1998) shown in table 5.1.

5.2.2.2 DIAGNOdent Analysis

An initial DIAGNOdent analysis of each specimen was undertaken and the peak DIAGNOdent value for the caries on the occlusal surface noted. Specimens were sectioned longitudinally through the carious lesion using a rotating diamond disc (Labcut 1010, Agar Scientific Ltd, D.R. Bennett Ltd, Leceister, England) (figure 5.28) and one half of the section mounted with its cut surface uppermost onto the stage of a travelling microscope (Model 7119, Swift, Basingstoke, England). Adhesive mounting compound (Tanwax Mounting Compound, Malvern Instruments, Malvern, England) was used to secure the specimen on the table. Using tan wax, the DIAGNOdent probe tip was attached rigidly onto the moveable telescope element of the travelling microscope (figure 5.29).

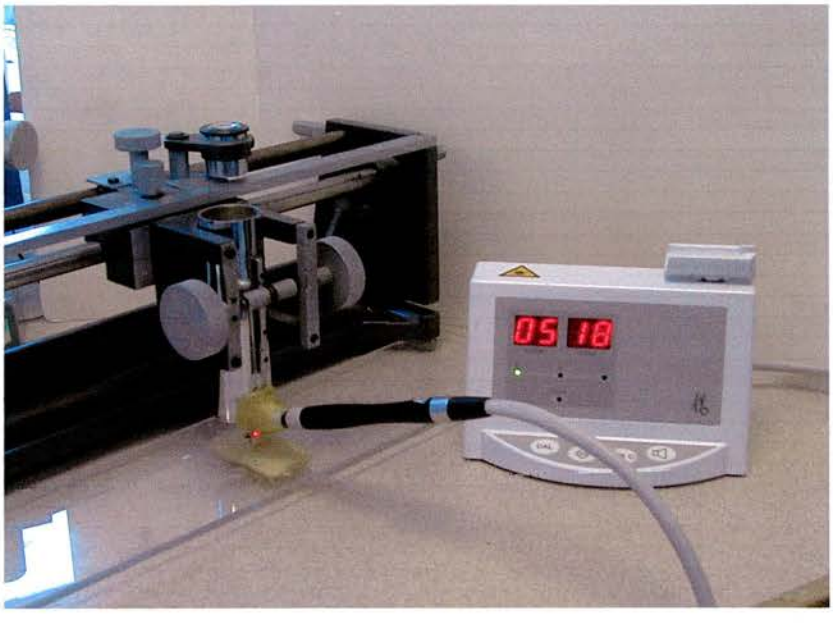
Figure 5.28 Longitudinally Sectioned Specimen Showing Carious Lesion

Figure 5.29 DIAGNOdent Probe Attached to Travelling Microscope



After zeroing the DIAGNOdent on an area of sound enamel on the specimen, the telescope and attached DIAGNOdent probe were carefully lowered to contact the cut surface of the longitudinally sectioned specimen. The movable gantry of the microscope was adjusted to position the probe tip directly over the occlusal surface of the carious lesion and the DIAGNOdent value at this point was recorded. (figure 5.30)

Figure 5.30 Travelling Microscope and DIAGNOdent Apparatus for Longitudinal Lesion Analysis



Using the mechanical gearing of the instrument, the position of the gantry was adjusted to move the DIAGNOdent probe tip over the surface of the specimen in an apical direction in 100μm increments. After each 100μm increment the

DIAGNOdent value at that point was recorded. Incremental movement and recording of DIAGNOdent value in this way was continued until the DIAGNOdent value returned below 9, the threshold for clinically significant caries.

5.2.2.3 Preparation of Sections for Microradiography

The specimen was removed from the bed of the travelling microscope and using a rotating diamond disc (Microslice 2 Precision Slicing Machine, Malvern Instruments, Malvern, England), (figure 5.27) a plano-parallel section of 300-500μm was cut from the surface. This section was then hand lapped in a slurry of Aluminium Oxide down to a thickness of between 120 and 140μm. The thickness of the section was confirmed with a micrometer (Mitutoyo Digimatic Indicator, Mitutoyo MFG Co. Ltd, Japan).

A 120 to 140μm section from the surface of the longitudinal section through each specimen was prepared in this way and carefully washed in de-ionised water and dried on filter paper. The sections were trimmed using a sharp blade to isolate the area of the carious lesion under study. With the aid of a template corresponding to the layout of the microradiography film cassette, the sections were mounted, in batches of 6 specimens, between two sheets of cling film. A reference diagram indicating the position of each specimen was completed to allow subsequent identification of each on the resultant microradiographs.

5.2.2.4 Transverse Microradiography

Under safe-light conditions, the cling film mounts (each containing 6 specimens) were placed, along with an aluminium step wedge of known thicknesses, onto X-ray sensitive film (Kodak SO 343 High Resolution Spectrographic Film, Eastman-Kodak Co., Rochester, NY, USA) and the film/specimen package loaded into light proof aluminium boxes (figure 5.31). In a Diffractis 582 X-ray machine (Diffractis 582, Diffractis, Delft, The Netherlands), these were exposed to Nickel filtered Cu(K_α) X-rays at 20kV and 30mA for 20 minutes with a target distance of 300mm (figure 5.32). Under safe-light conditions, the films were removed and developed using standard chemistry.

Figure 5.31 Light-Proof Boxes for Exposing Contact Microradiographs

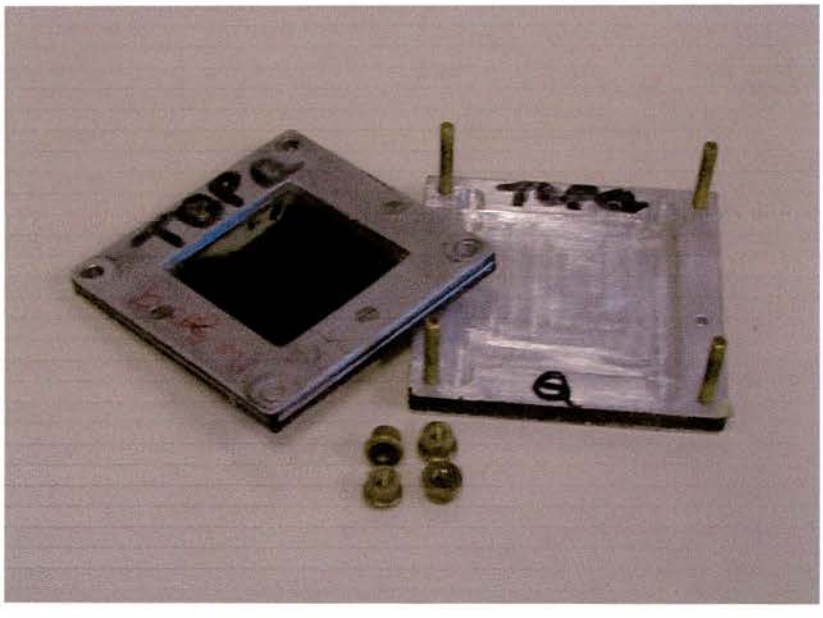
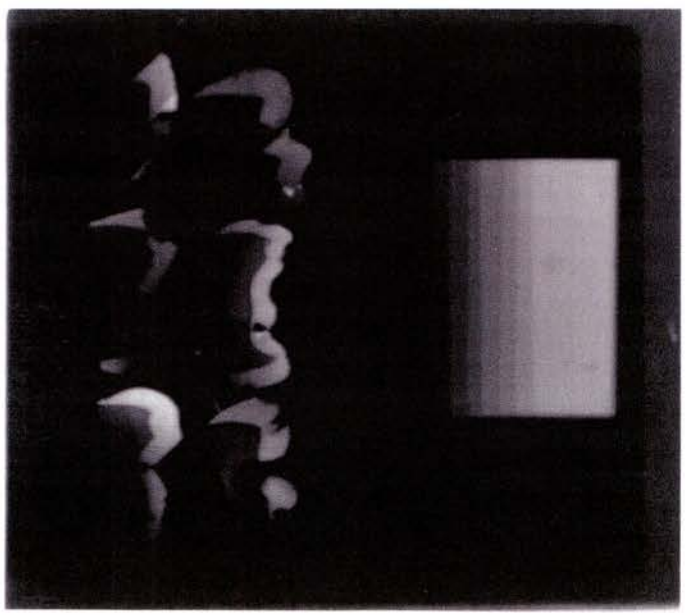


Figure 5.32 Diffractis 582 X-ray Machine

The resultant microradiographs were dried and taken for microdensitometrical analysis. Microradiographs of specimens 1-6 along with the calibration stepwedge are illustrated in figure 5.33.

Figure 5.33 Transverse Microradiographs of Specimens 1-6



5.2.2.5 Densitometric Analysis

The microradiographs were placed on the stage of a light microscope (Leitz Ortholux, Leitz, Wetzlar, Germany) and illuminated with a stabilised illumination system (Voltage Stabiliser CL 6123). An image of the lesion was recorded using a CCD video camera (Cohu High performance CCD Camera, Cohu, CA, USA) and anlysed using a microcomputer loaded with densitometric software (Transverse Micro Radiography v1.25e, Inspektor Research Systems BV, Amsterdam, The Netherlands). The apparatus for densitometry is shown in figure 5.34

Figure 5.34 Apparatus for Densitometric Analysis

The densitometry software was calibrated by registering, in turn, each zone of the image of the aluminium step wedge. The computer was then able to construct a calibration curve relating the stepwedge thickness to the light transmission through the film. This calibration curve was then used automatically by the software for analysis of each lesion on that particular microradiograph. The area of each lesion to be analysed was selected by using the computer mouse to enclose a portion of the displayed image of the lesion. The software then digitised the light transmission at each point within this enclosed area to 256 grey levels and by applying the equation derived by Angmar et al., (1963), was able to compute the percentage mineral volume from the recorded grey level. Using the previously derived calibration curve, a plot of sample position (μm) against mineral volume (%) for the selected lesion area was generated.

5.2.3 Results

5.2.3.1 Distribution of Fluorescence

The DIAGNOdent value at each point within the lesion was plotted against the distance into the lesion from the base of the fissure. Plots for each specimen are shown in figures 5.35 - 5.58.

Figure 5.35 Plotted DIAGNOdent Values within Lesion (Specimen 1)

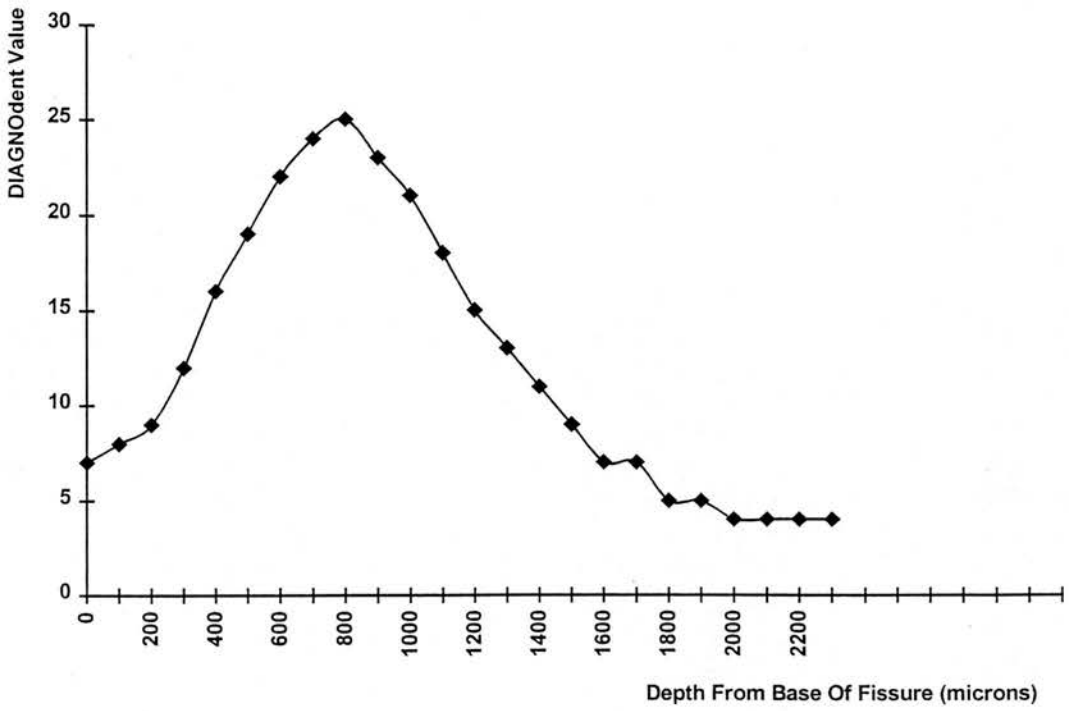


Figure 5.36 Plotted DIAGNOdent Values within Lesion (Specimen 2)

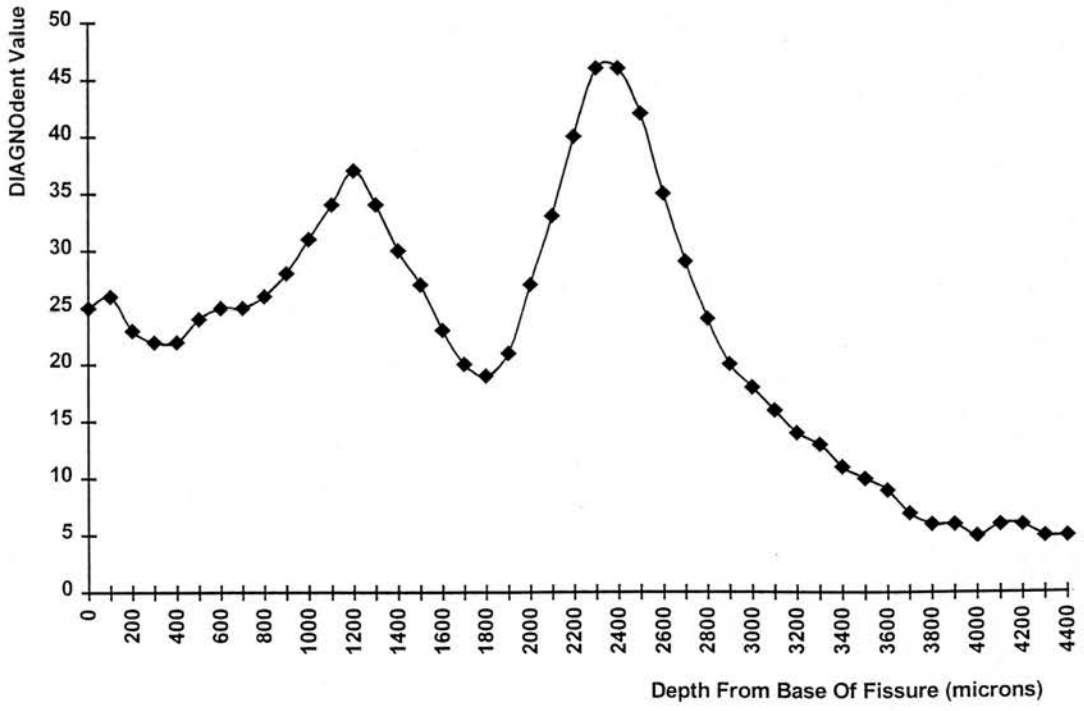


Figure 5.37 Plotted DIAGNOdent Values within Lesion (Specimen 3)

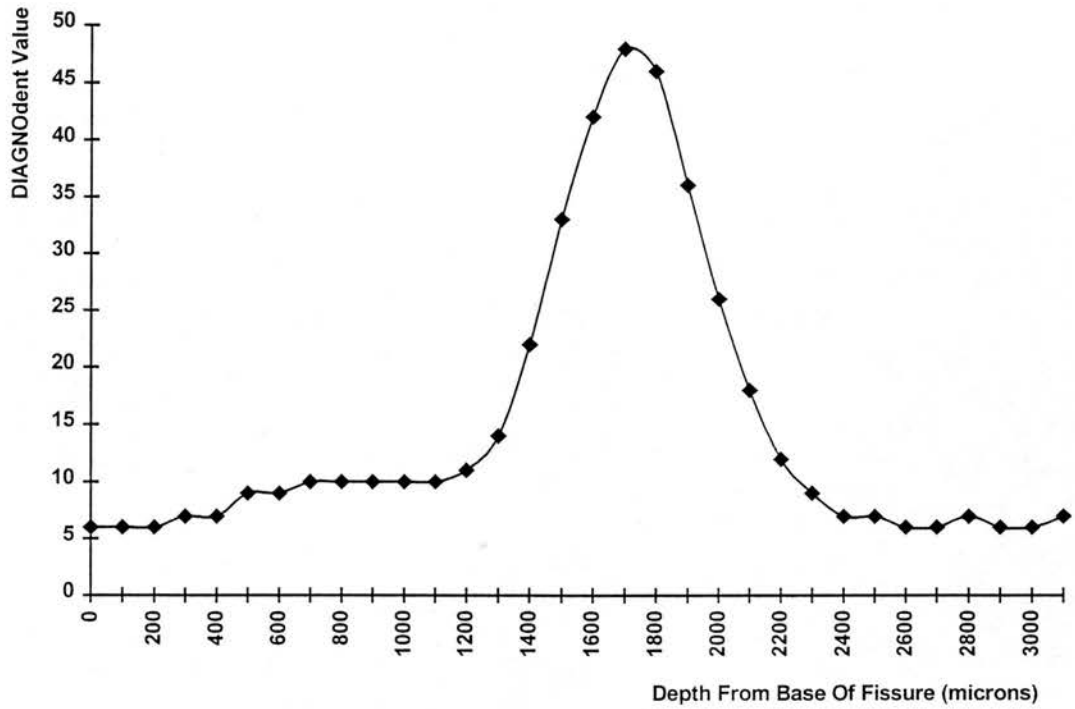


Figure 5.38 Plotted DIAGNOdent Values within Lesion (Specimen 4)

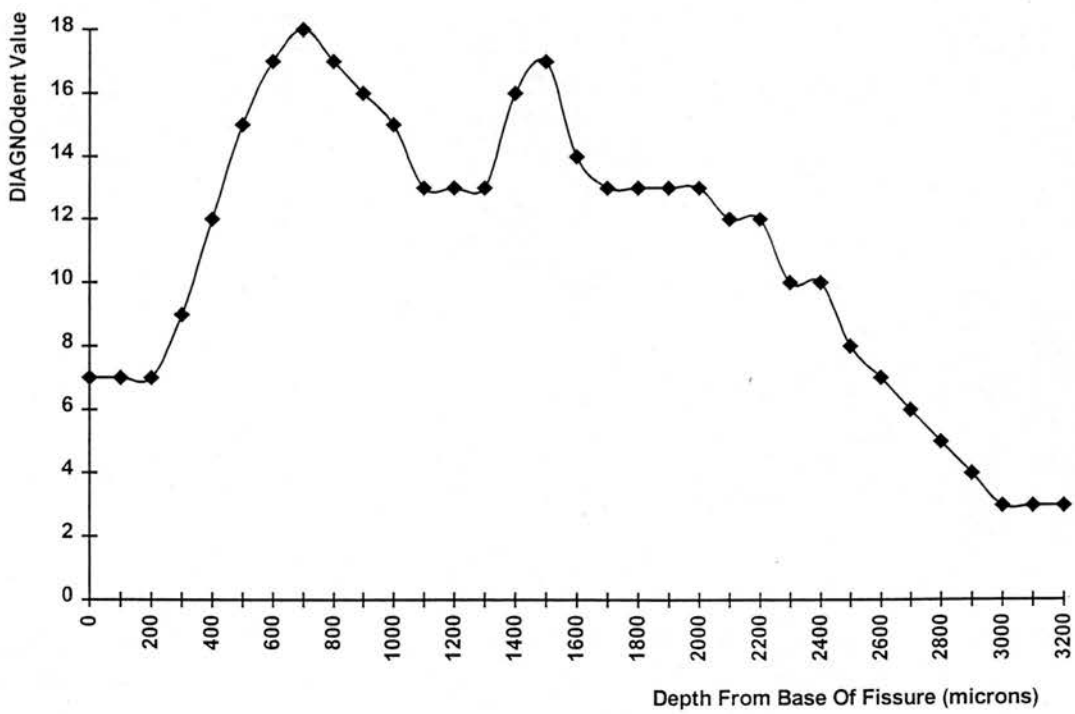


Figure 5.39 Plotted DIAGNOdent Values within Lesion (Specimen 5)

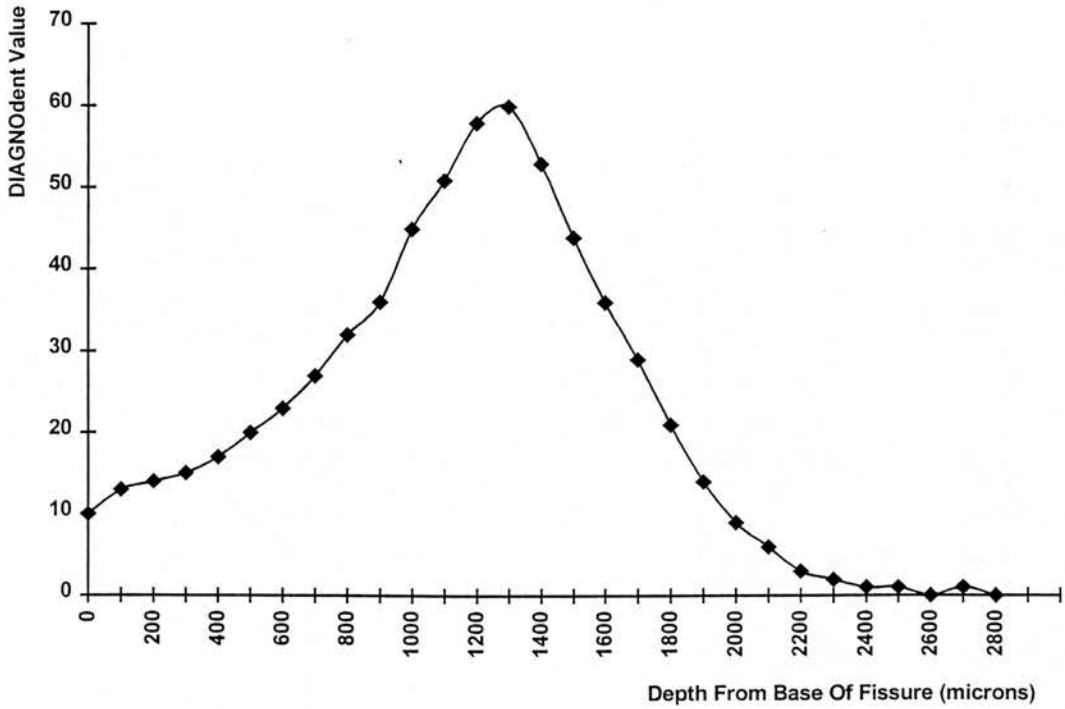


Figure 5.40 Plotted DIAGNOdent Values within Lesion (Specimen 6)

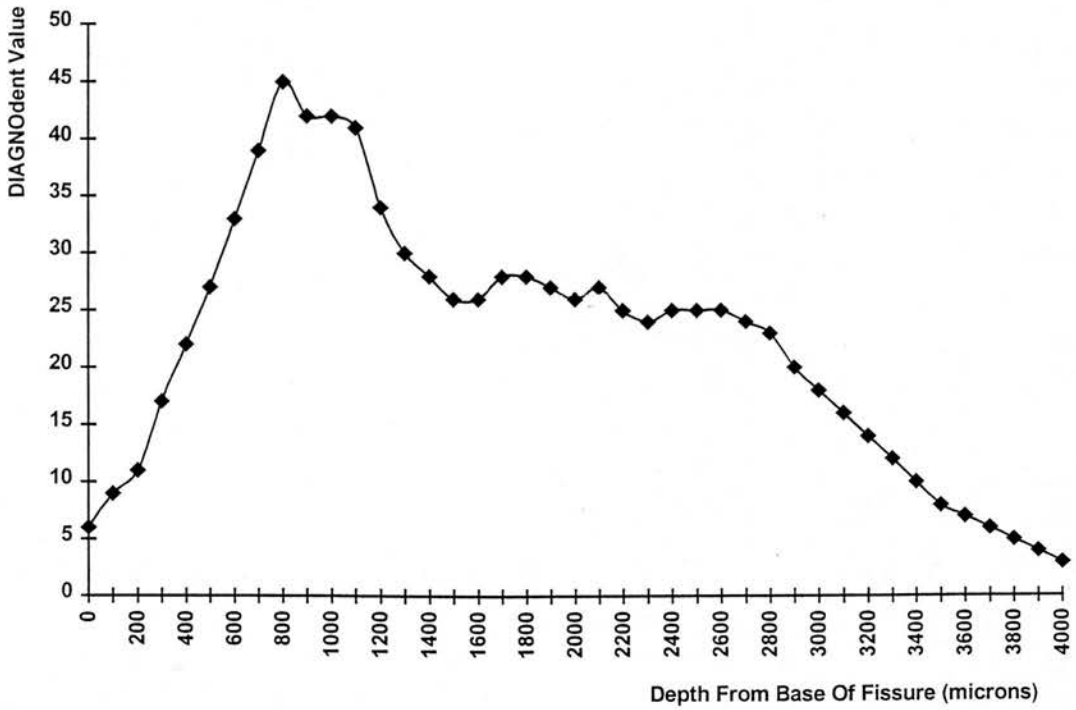


Figure 5.41 Plotted DIAGNOdent Values within Lesion (Specimen 7)

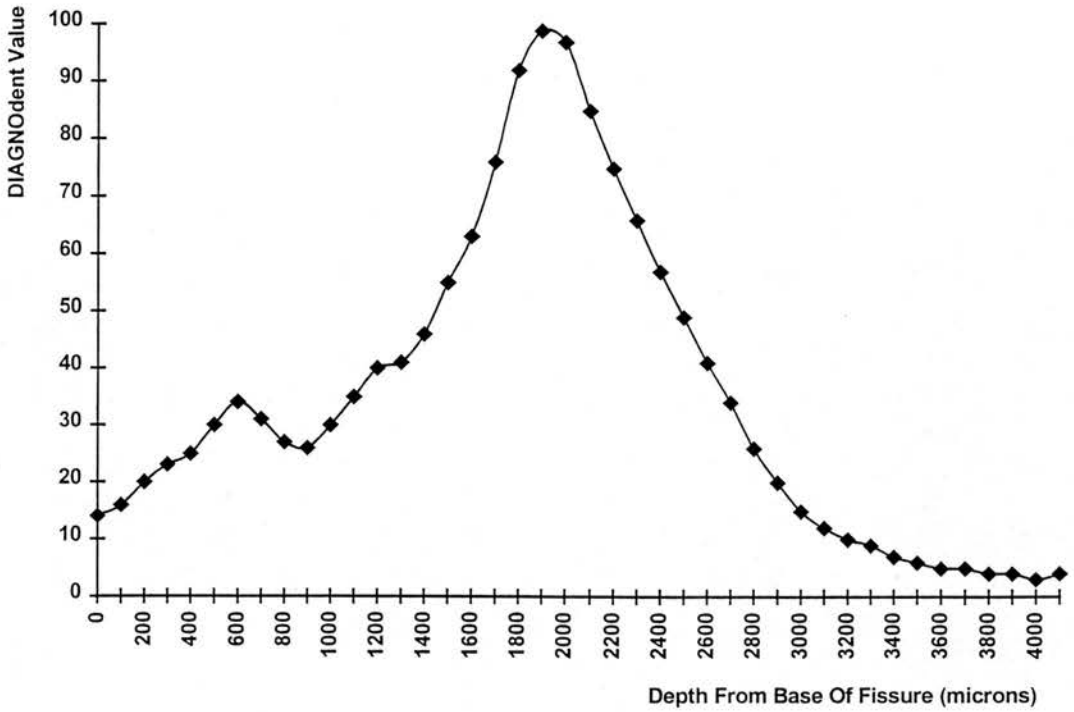


Figure 5.42 Plotted DIAGNOdent Values within Lesion (Specimen 8)

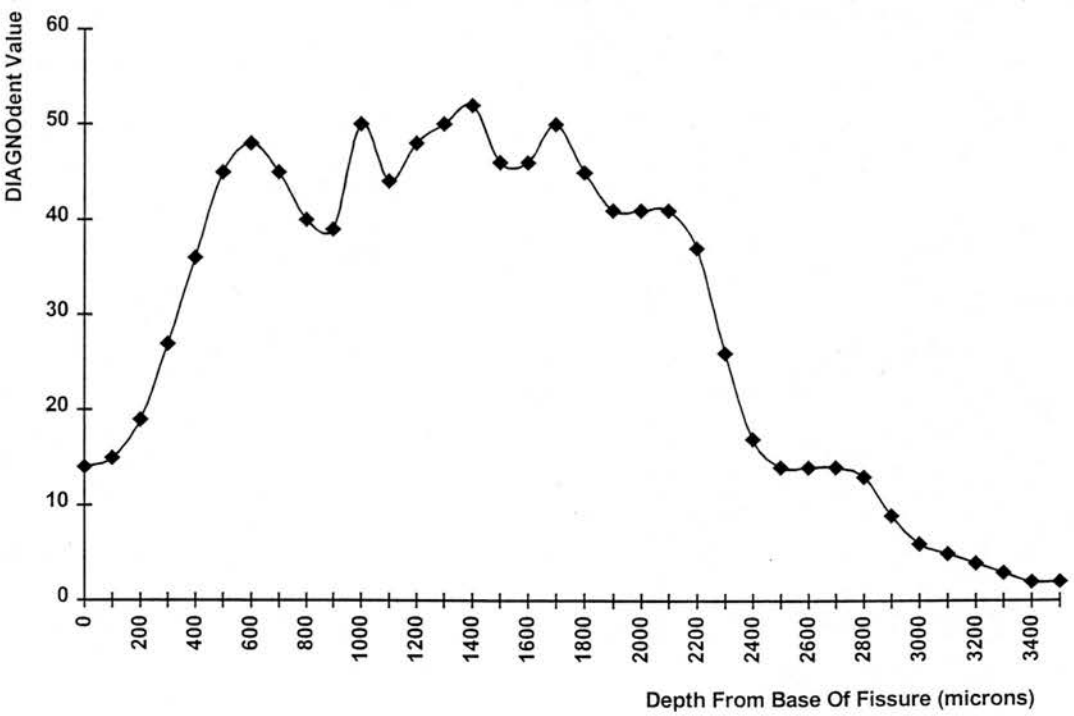


Figure 5.43 Plotted DIAGNOdent Values within Lesion (Specimen 9)

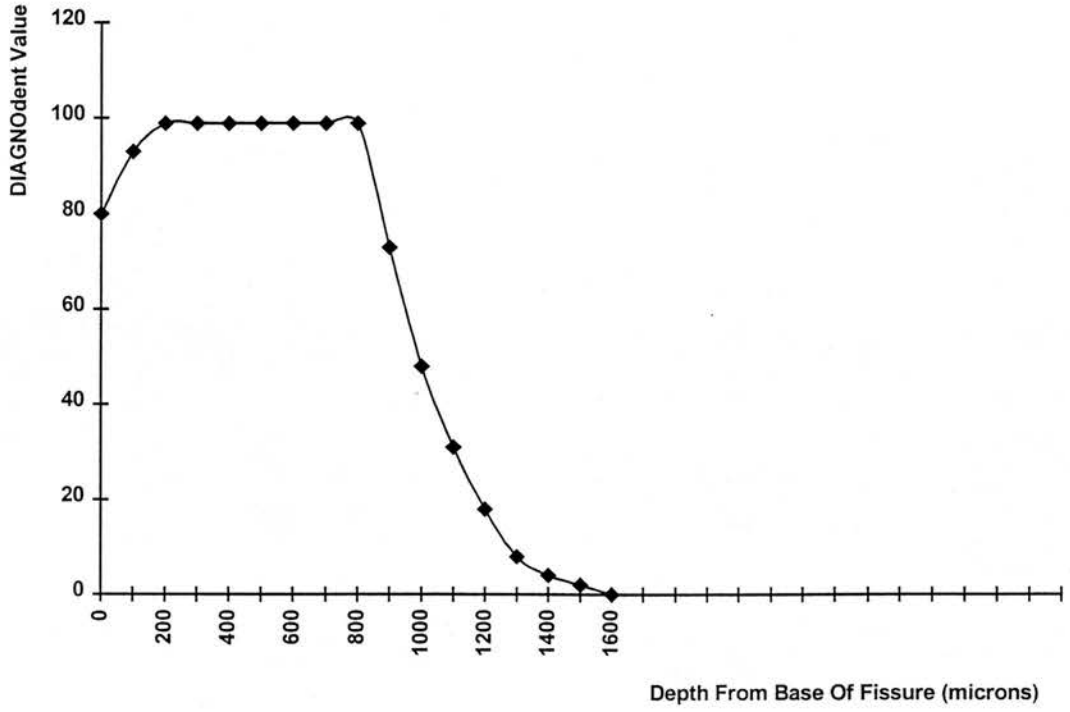


Figure 5.44 Plotted DIAGNOdent Values within Lesion (Specimen 10)

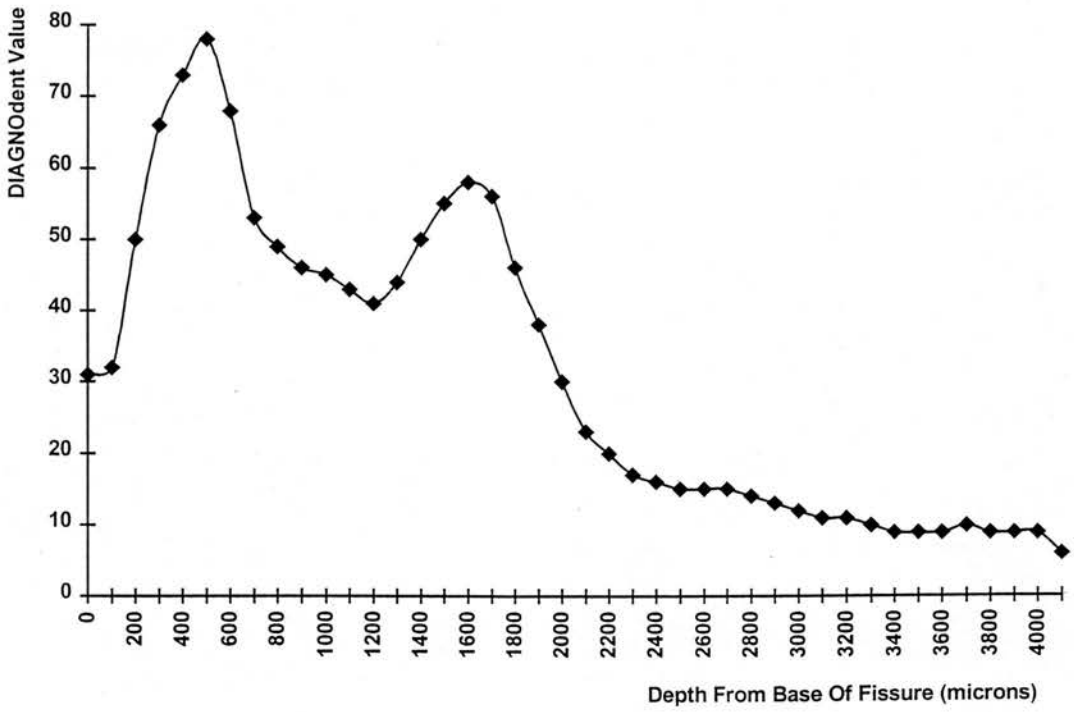


Figure 5.45 Plotted DIAGNOdent Values within Lesion (Specimen 11)

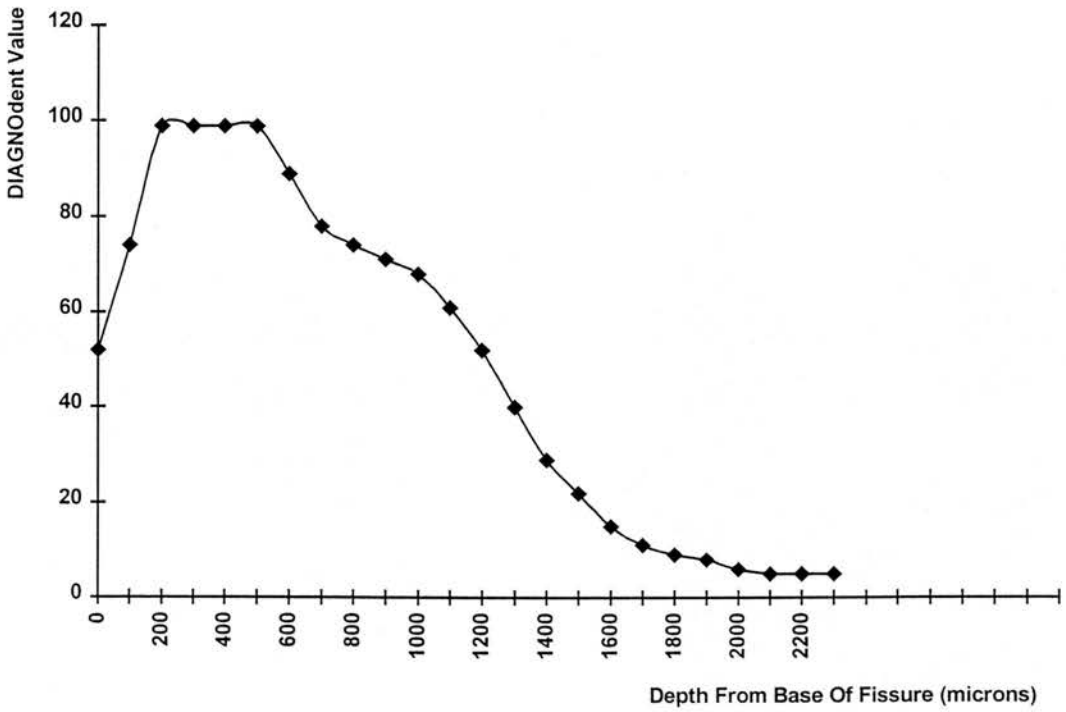


Figure 5.46 Plotted DIAGNOdent Values within Lesion (Specimen 12)

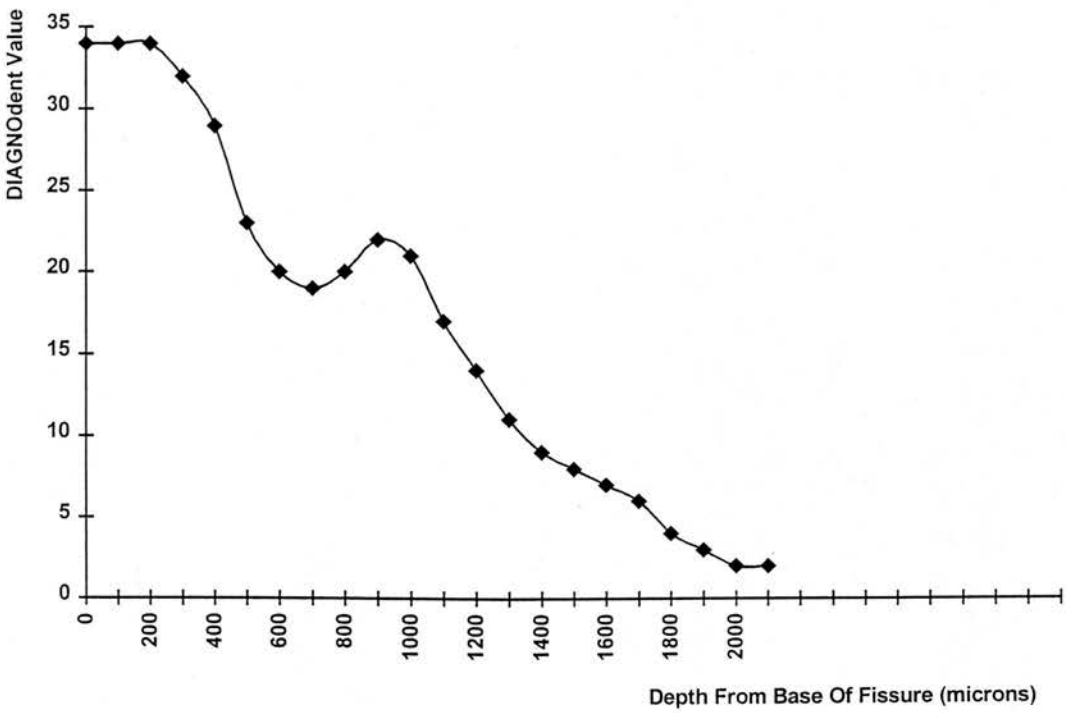


Figure 5.47 Plotted DIAGNOdent Values within Lesion (Specimen 13)

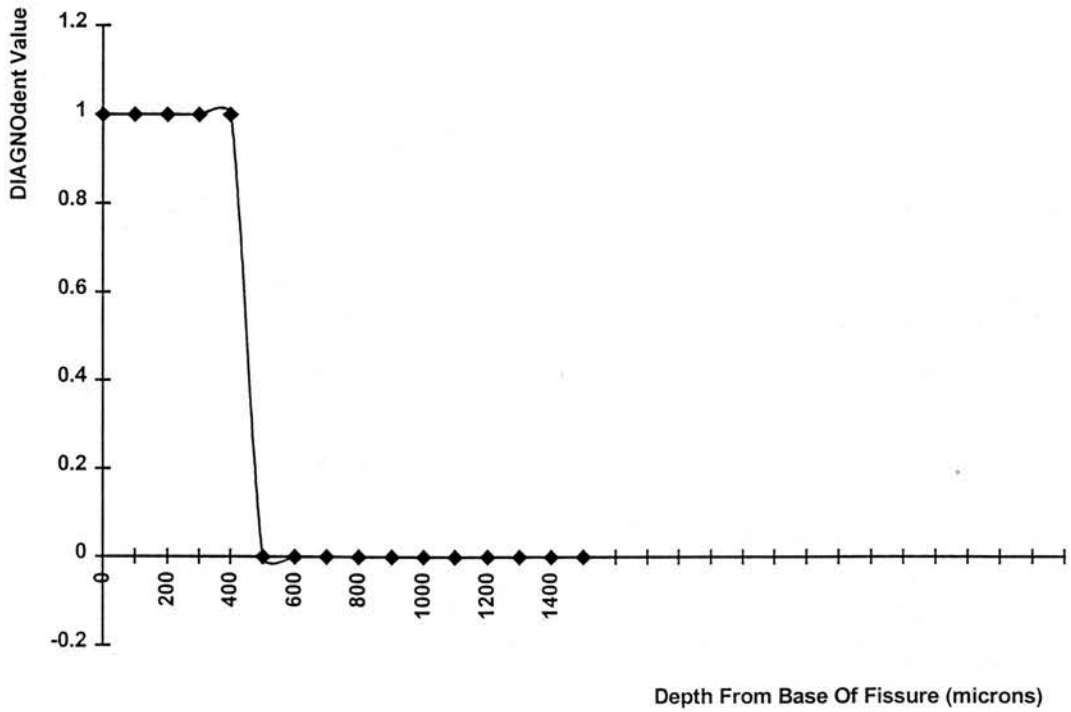


Figure 5.48 Plotted DIAGNOdent Values within Lesion (Specimen 14)

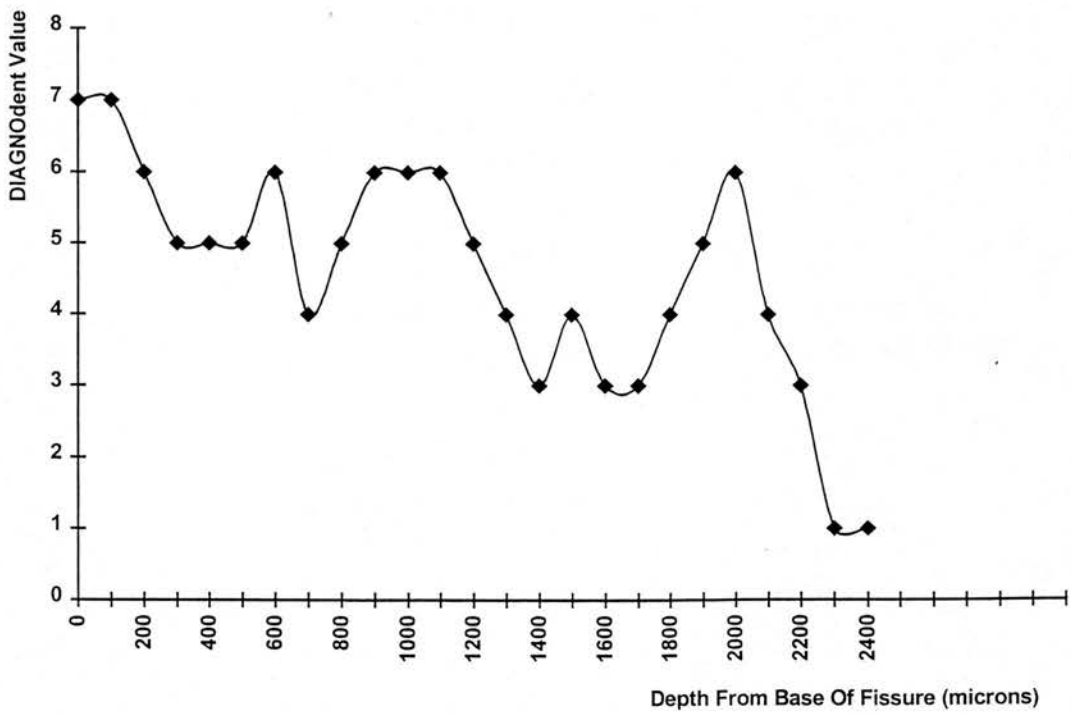


Figure 5.49 Plotted DIAGNOdent Values within Lesion (Specimen 15)

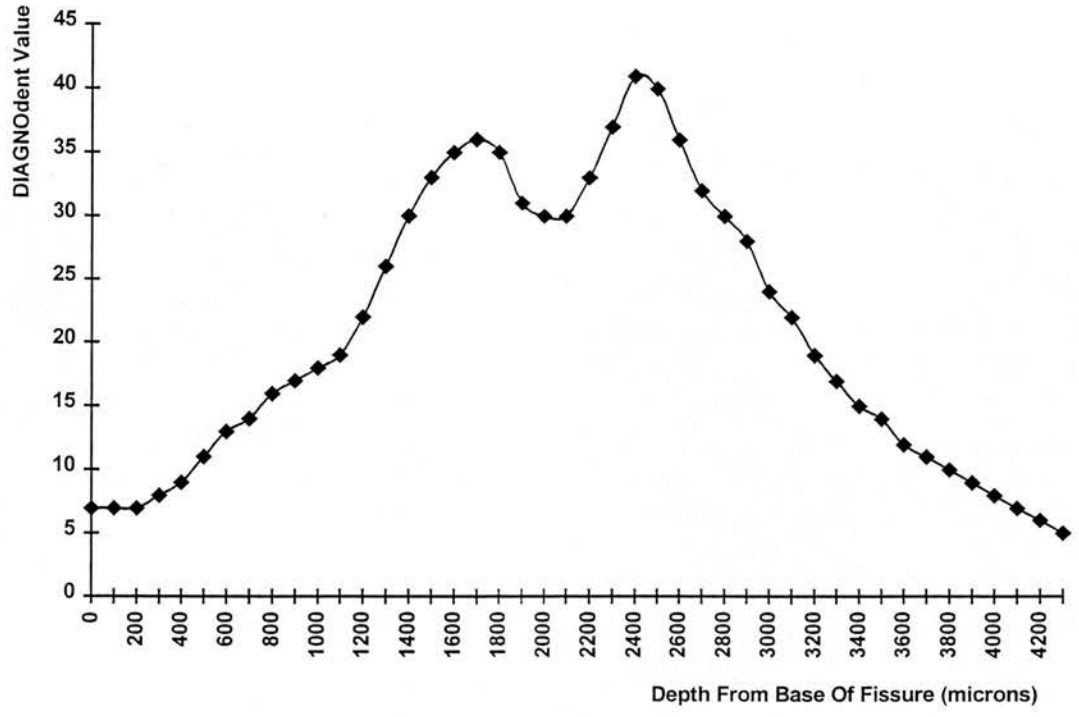


Figure 5.50 Plotted DIAGNOdent Values within Lesion (Specimen 16)

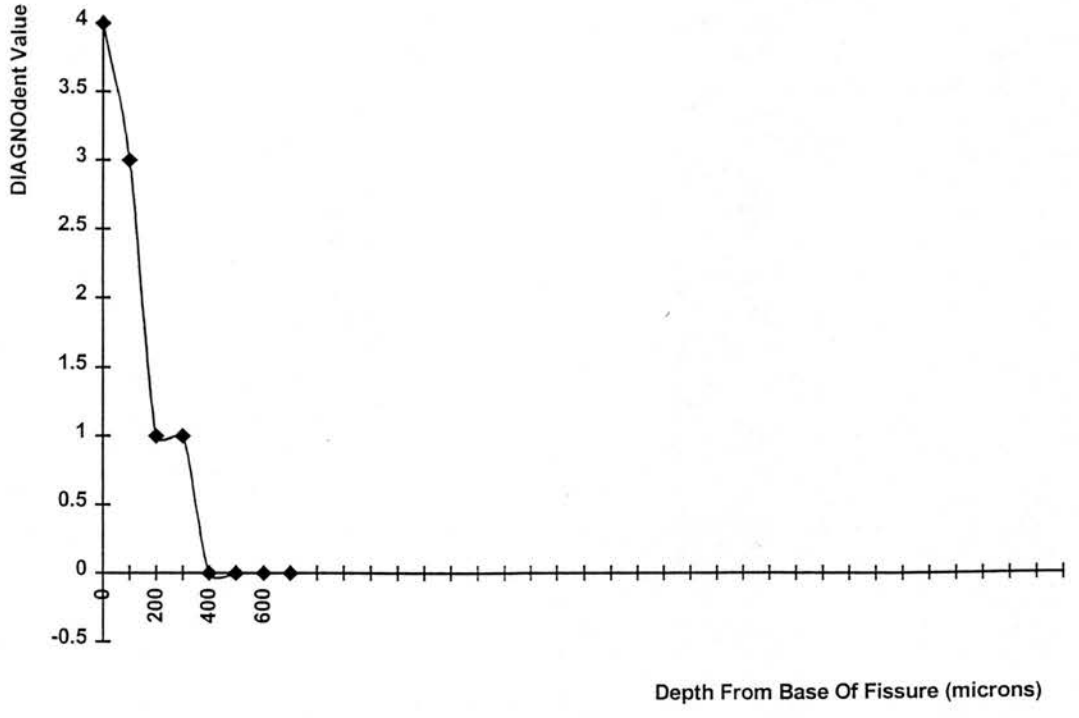


Figure 5.51 Plotted DIAGNOdent Values within Lesion (Specimen 17)

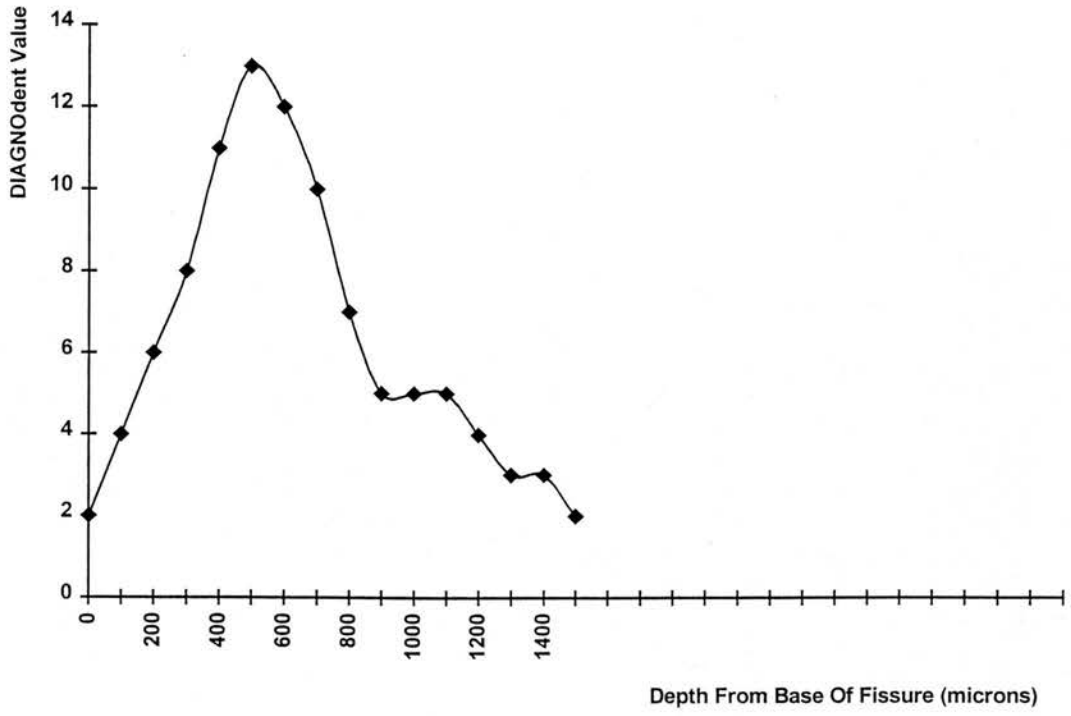


Figure 5.52 Plotted DIAGNOdent Values within Lesion (Specimen 18)

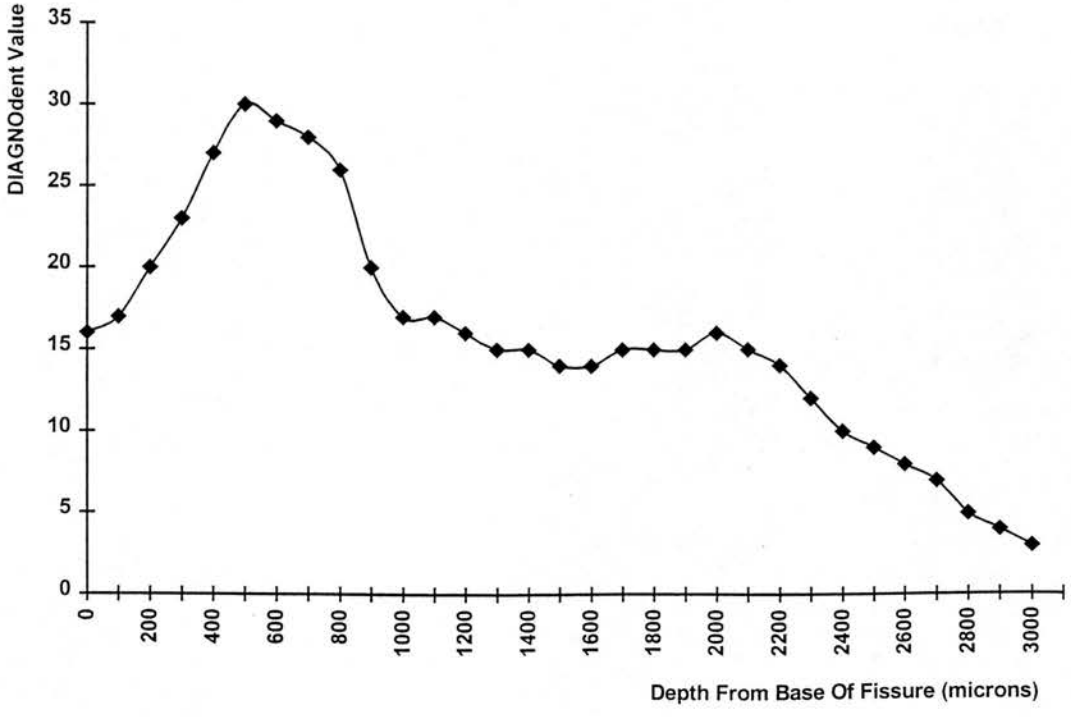


Figure 5.53 Plotted DIAGNOdent Values within Lesion (Specimen 19)

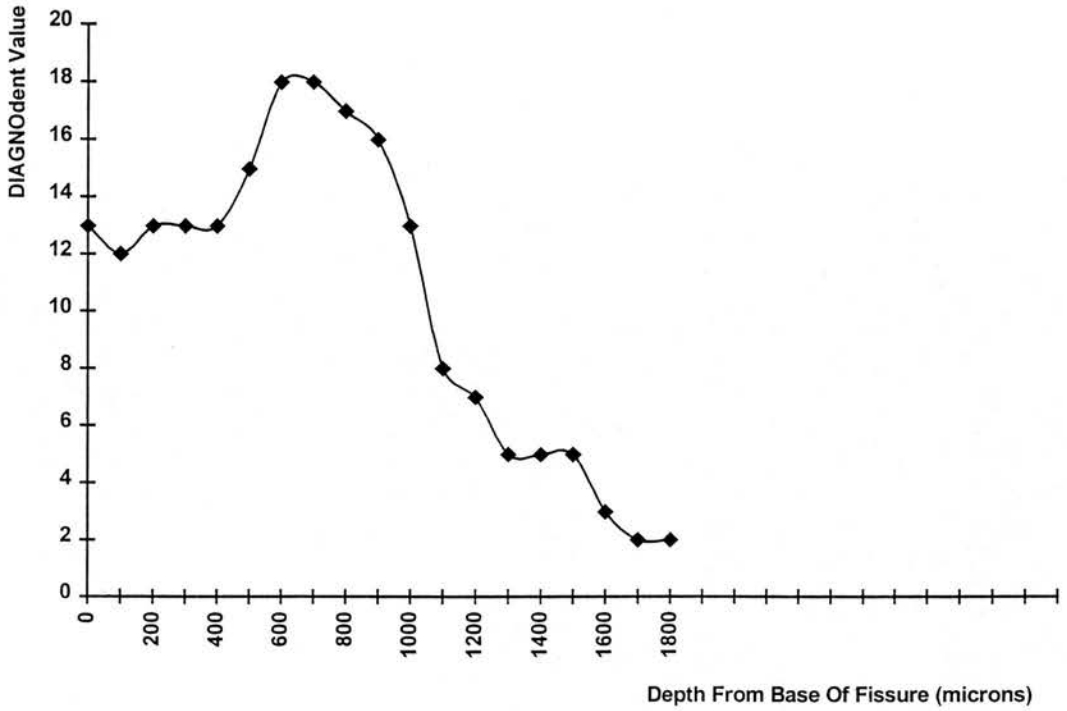


Figure 5.54 Plotted DIAGNOdent Values within Lesion (Specimen 20)

Figure 5.55 Plotted DIAGNOdent Values within Lesion (Specimen 21)

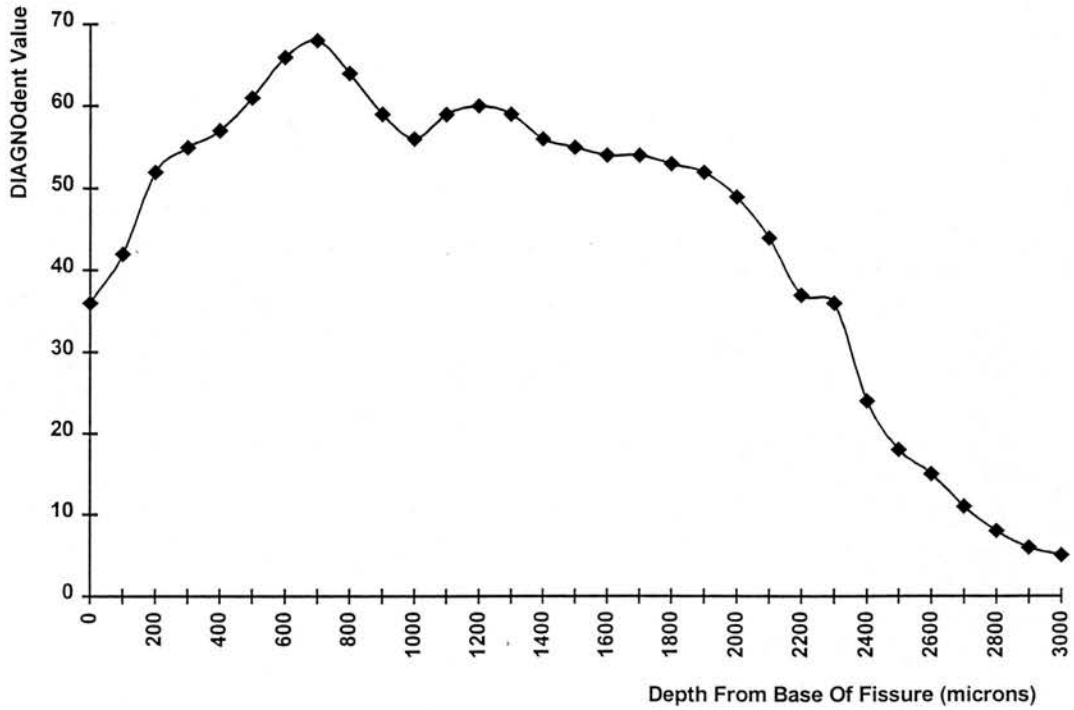


Figure 5.56 Plotted DIAGNOdent Values within Lesion (Specimen 22)

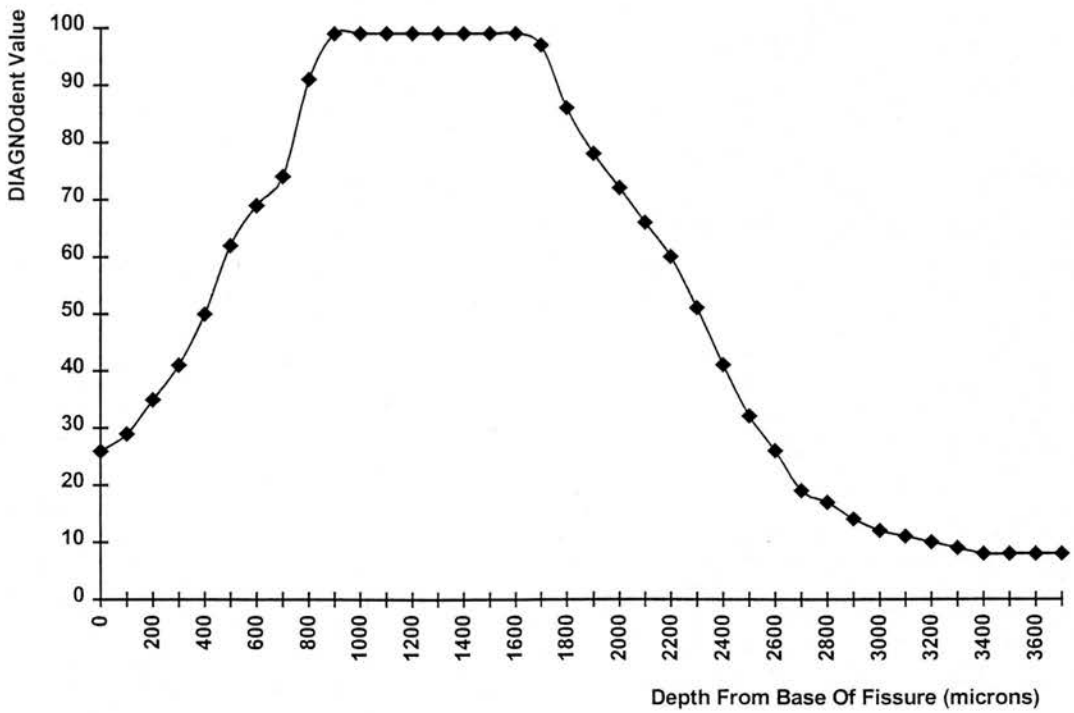


Figure 5.57 Plotted DIAGNOdent Values within Lesion (Specimen 23)

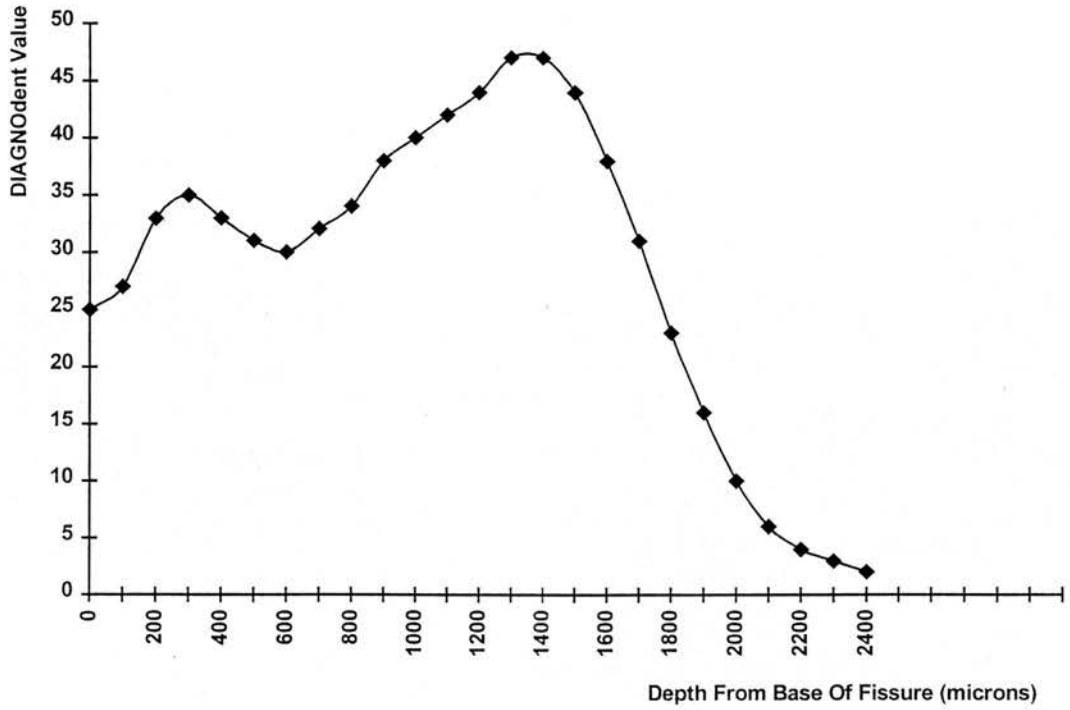
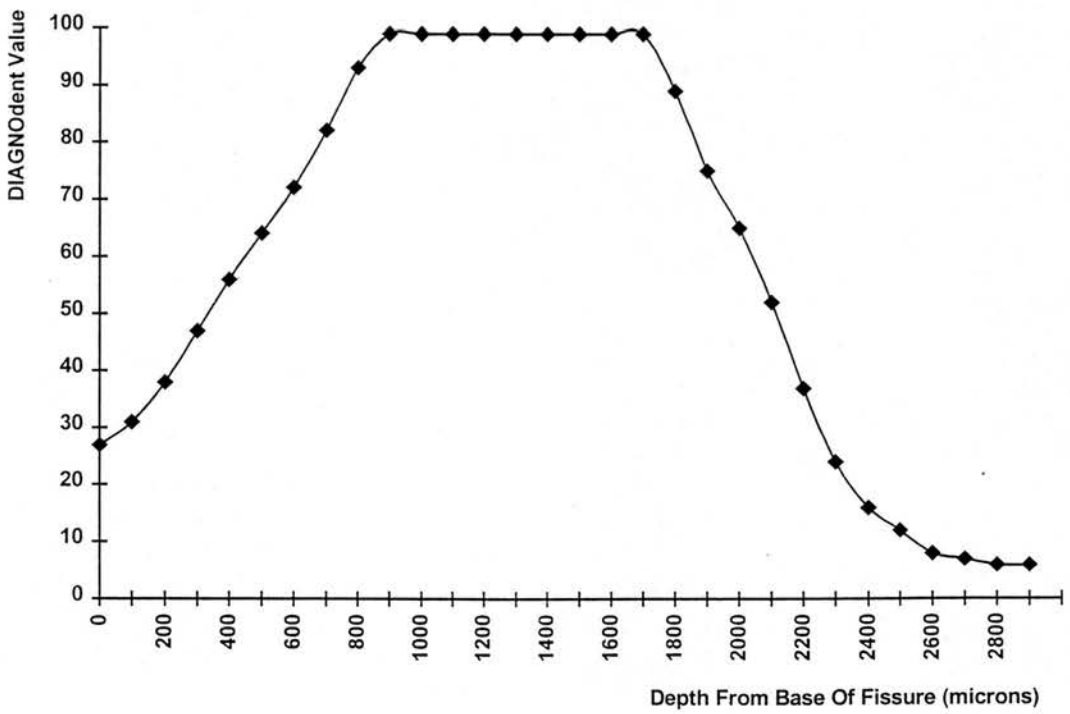


Figure 5.58 Plotted DIAGNOdent Values within Lesion (Specimen 24)



5.2.3.2 Correlation between Visual and DIAGNOdent Assessment of Lesions

The initial DIAGNOdent value for each intact lesion prior to any alteration by grinding of the occlusal surface was recorded. In addition a mean DIAGNOdent value for each specimen was determined by calculating the mean of all readings obtained during subsequent lesion dissection. From the plots of DIAGNOdent value against depth from the base of the fissure (DDV plot), the peak value for each specimen recorded during the stepwise analysis was noted along with the depth at which the DIAGNOdent value fell below the threshold level of 9. Furthermore, the area under the DDV plot to this point (or to the point of pulp exposure) was calculated for each individual lesion. The above data are shown for each specimen in tables 5.5-5.7. These data were studied for correlation between the recorded parameters and correlation co-efficients are shown in table 5.8.

Table 5.5. Summary Data for DIAGNOdent Analysis of Longitudinal Dissection of Lesions (Specimens 1-8)

Specimen No.	Clinical Caries Score	Initial DIAGNOdent Value	Area under DDV Curve	Mean DIAGNOdent Value	Peak DIAGNOdent Value	Lesion Depth
1	2a	32	25200	12.2	25	1600
2	2a	37	92050	22.2	46	3700
3	2a	34	41750	14.7	48	2400
4	2a	20	32200	10.9	18	2500
5	2a	23	62500	22.1	60	2100
6	3	25	86700	22.1	45	3500
7	1a	29	139400	34.7	99	3400
8	4	54	101750	29.1	52	3000

Table 5.6. Summary Data for DIAGNOdent Analysis of Longitudinal Dissection of Lesions (Specimens 9-16)

Specimen No.	Clinical Caries Score	Initial DIAGNOdent Value	Area under DDV Curve	Mean DIAGNOdent Value	Peak DIAGNOdent Value	Lesion Depth
9	1a	99	100000	61.8	99	1300
10	1	16	128800	31.3	78	4100
11	2	47	111900	48.8	99	1900
12	3	49	32600	16.9	34	1500
13	3	33	0	0.3	1	0
14	4	14	0	4.6	7	0
15	1a	17	86800	20.7	41	4000
16	4	70	0	1.1	4	0

Table 5.7. Summary Data for DIAGNOdent Analysis of Longitudinal Dissection of Lesions (Specimens 17-24)

Specimen No.	Clinical Caries Score	Initial DIAGNOdent Value	Area under DDV Curve	Mean DIAGNOdent Value	Peak DIAGNOdent Value	Lesion Depth
17	1	27	6850	6.3	13	800
18	1	9	46000	15.9	30	2600
19	3	73	15850	10.4	18	1100
20	1	24	14000	12.0	25	1000
21	4	99	133200	44.2	68	2800
22	2a	85	194900	52.7	99	3400
23	3	49	69050	28.6	47	2100
24	2a	44	175900	59.9	99	2600

Table 5.8. Correlations between Recorded Parameters in Longitudinal Lesion Dissection

Parameters	Spearman's Rank Correlation Coefficient
Clinical Caries Score vs Area under DDV Curve	0.11
Clinical Caries Score vs Mean DIAGNOdent Value	0.15
Clinical Caries Score vs Initial DIAGNOdent Value	0.34
Clinical Caries Score vs Lesion Depth	-0.20
Clinical Caries Score vs Peak DIAGNOdent Value	0.06
Initial DIAGNOdent Value vs Area under DDV Curve	0.28
Initial DIAGNOdent Value vs Mean DIAGNOdent Value	0.37
Initial DIAGNOdent Value vs Lesion Depth	-0.14
Initial DIAGNOdent Value vs Peak DIAGNOdent value	0.31
Peak DIAGNOdent Value vs Mean DIAGNOdent Value	0.96*
Peak DIAGNOdent Value vs Lesion Depth	0.59*
Peak DIAGNOdent Value vs Area under DDV curve	0.93*
Mean DIAGNOdent Value vs Lesion Depth	0.61*
Mean DIAGNOdent Value vs Area under DDV Curve	0.95*

* denotes statistical significance at $p<0.05$ level.

5.2.4 Discussion

5.2.4.1 Practical Considerations

In practice, the precise location of the DIAGNOdent probe tip to commence lesion analysis was difficult. Following tooth sectioning, it was intended to begin the DIAGNOdent analysis at the same point at the base of the fissure where the initial pre-sectioning analysis was carried out. Whilst care was taken to accurately position the probe tip over the exact point, the possibility for inconsistency was acknowledged. Given the variation in recorded DIAGNOdent values over as small a distance as 100μm, a small discrepancy in the starting position of the analysis may have been a significant source of error. This may have some part to play in explaining the variation between the initial DIAGNOdent value recorded from the unaltered occlusal surface and the subsequent value at the starting point of the analysis of the longitudinal section.

The area of tissue coverage of the light source of the DIAGNOdent has not been defined. The penetration and spread of the light would be expected to vary with the optical density of the tissue under study as well as with the characteristics of the light source and the physical dimensions of the probe tip. The narrower 'A' probe tip was used for the analyses in this experiment but as previously discussed in 5.1.4.1, it is not known whether the 100μm increment was beyond the spatial descriminatory ability of the DIAGNOdent in this configuration.

5.2.4.2 Pattern of Fluorescence

All specimens selected for this experiment demonstrated visual evidence of occlusal caries and subsequently all specimens, on initial DIAGNOdent examination, demonstrated a value greater than or equal to 9 which is indicative of clinically significant caries. However, following longitudinal sectioning through the lesion, 3 specimens (nos. 13, 14 and 16) failed to demonstrate clinically significant caries on subsequent DIAGNOdent analysis of the cut surface. A number of other lesions subjectively appeared to demonstrate very minimal carious involvement beneath the outermost enamel surface and would have, on this basis, been inappropriate for invasive restoration. This would apparently support the previously proposed theory (section 3.3) that the DIAGNOdent value is prone to influence from surface staining phenomena and this may result in misdiagnosis of caries.

This is indicative of the difficulty of diagnosing the true carious status of an occlusal surface even on clean, dry, extracted teeth. In the clinical situation, the difficulty is further compounded. A number of authors have focused on this problem of 'hidden' caries where there is little or no sign of demineralisation on the occlusal surface, but extensive demineralisation is evident radiographically or on operative investigation (Creanor et al., 1990; Pitts and Deery 1994). The results of DIAGNOdent analysis of the occlusal lesions in this study would appear to confirm this phenomenon with visually sound or minimally altered occlusal surface subsequently demonstrating extensive lesion fluorescence.

Subjective analysis of DIAGNOdent values plotted against lesion depth for individual specimens was more indicative of a pattern than similar plots for the data from the initial lesion dissection experiment (section 5.1). Most specimens exhibited a rise to a peak DIAGNOdent value within the lesion falling gradually down below the threshold for clinically significant caries as the analysis moved through the base of the lesion and into the sound dentine beneath. Nevertheless, within this trend there existed considerable variation and it was not possible to demonstrate a consistent pattern of fluorescence within these lesions.

5.2.4.3 Correlation of DIAGNOdent Analysis with Other Parameters

In the previous experiment employing incremental removal of the lesion surface (section 5.1), no significant correlations were demonstrated between the clinical caries score, the initial DIAGNOdent value and the other parameters. This method involving direct DIAGNOdent measurement of a longitudinal tooth section also failed to demonstrate any significant correlations between clinical caries score, DIAGNOdent value and any of the other parameters under study. This experiment therefore, would also seem to suggest that the DIAGNOdent value of occlusal caries in non-cavitated lesions is a poor indicator of the true extent of the caries in that tooth. However, as in the stepwise lesion dissection described in Section 5.1, significant correlations were seen between peak and mean DIAGNOdent values, peak DIAGNOdent and lesion depth and peak DIAGNOdent value and area under

the DDV curve. Furthermore, the mean DIAGNOdent value correlated significantly with lesion depth and area under the DDV curve.

In comparison, Ekstrand et al., 1997 investigated the ability of visual scoring, radiography and an electronic caries detection system to detect occlusal caries and to predict the depth of the lesion. This *in vivo* study demonstrated excellent correlations (r_s=0.75 for the visual scoring system) between these diagnostic methods and the true depth of the lesion as confirmed histologically. Other studies by Ekstrand et al., (1998) have also demonstrated the validity of the visual scoring system as a means of predicting the extent of a lesion and the lack of correlation between the clinical caries score and the lesion depth found in this study would question the validity of the DIAGNOdent to accurately measure lesion depth when employed in the technique described here.

5.2.4.4 Transverse Microradiography

Microdensitometrical analysis of the microradiographs of these lesions proved difficult. A number of microradiographs demonstrated significant preparation artefacts and the extent and variability of the lesions were not well suited to this analytical technique. It was attempted to construct a profile of mineral loss through the lesion by selecting for analysis a narrow zone passing through the base of the lesions which corresponded to the zone which had been scanned by the DIAGNOdent. However, the microradiographs of a number of the specimens did not

allow identification of a suitable zone. A direct comparison of fluorescence as measured by the DIAGNOdent with mineral loss as measured by microdensitometry for each lesion was highly desirable but not achievable in practice.

CHAPTER 6

SUMMARY AND CONCLUSIONS

6.1 Summary of Experimental Work

This thesis describes work which was undertaken to evaluate the DIAGNOdent caries detection system. A series of individual but linked experiments was carried out with the results of each individual experiment raising questions which influenced the direction and design of the subsequent work.

Initially the investigation assessed the ability of the device to detect not caries but demineralisation *per se*. Artificial carious lesions were created in enamel specimens derived from extracted human teeth. Different lesion creation protocols were employed to investigate the effects of enamel surface abrasion and the use of two different demineralising solutions. A total of 220 artificial lesions and control specimens were analysed by the DIAGNOdent device and the ability of the DIAGNOdent to reflect the extent of demineralisation assessed.

Although analysis of results indicated a significant correlation between DIAGNOdent value and demineralisation exposure time, the actual correlation value was low and in practical usage, the device was subjectively unconvincing in its ability in this respect. The same artificial lesions were also analysed using another fluorescence - based caries detection device, the QLF \ clin. This device was better able to detect the differing extents of demineralisation and the values of correlation between measured QLF parameters and demineralisation exposure time were higher.

Also investigated at this stage were the intra and inter-examiner agreements for DIAGNOdent analysis. This was established by each of 4 individual examiners carrying out repeat analyses of each specimen. In this way comparison of measurements by the same examiner and also between different examiners was possible. Acceptable intra and inter examiner agreements, comparable with those reported elsewhere were demonstrated.

During this initial experiment, it was observed that the actual values recorded in DIAGNOdent analysis of the artificial carious lesions were much lower than those obtained during examination of natural carious lesions during clinical use of the device. In addition, the influence of stained tooth surfaces and surface deposits of calculus have been noted when using the DIAGNOdent clinically. It was therefore proposed to investigate the effect of staining on the operation of the DIAGNOdent device.

Initial investigations were made into developing a suitable staining medium and various substances including tea, coffee and red wine were investigated. It was apparent that tea stain had the most easily measurable effect and, in addition, this was considered to be a clinically relevant material. The 220 artificial lesions, which were initially created for DIAGNOdent analysis, were stained, by immersion in tea solution, for 24 hours. The lesions were noticeably stained at this point and a repeat of all DIAGNOdent and QLF analyses was undertaken to determine the effect of the staining on the results.

As a result of introducing the stain into the lesions, the recorded DIAGNOdent values were noticeably increased in comparison to analysis of the same lesions prior to staining. The ability of the device to distinguish between the different extents of demineralisation was also improved, although it was noted that there was an obvious difference in this respect between the results of examiners. This difference was seen between analysis of the lesions immediately upon removal from the stain and analysis after a delay of 7 days.

This observation raised the possibility of an instability in the effect which the exposure to the tea staining solution appeared to have exerted. However, although all other factors in the analyses carried out by the different examiners were constant, it was considered appropriate to investigate this potential instability further. A further group of artificial carious lesions was created and these specimens stained according to the same protocol as before. As with the initial group of lesions, the DIAGNOdent values obtained in analysis were significantly increased following staining and in addition, these values were seen to gradually decline to a new elevated baseline during the subsequent monitoring period. This appeared to confirm that not only was the DIAGNOdent assessment of artificial carious lesions susceptible to alteration, but this alteration was unstable over a given period of time.

This observed alteration in DIAGNOdent assessment of demineralisation as a result of staining was considered a potential source of uncertainty in the clinical application of the device as naturally occurring demineralised areas on tooth surfaces *in vivo*

would be exposed to staining materials in food and drinks. However, it is not possible to assume identical behaviour in natural caries and accordingly, a further experiment was undertaken to establish the effect of introducing stain into naturally occurring carious lesions. Extracted molar teeth with non-cavitated occlusal caries were examined using the DIAGNOdent device prior to exposing the teeth to the same staining protocol employed previously. As with the artificially created lesions, the natural lesions appeared to take up stain and a significant increase in DIAGNOdent value was recorded before and after the period of staining. Furthermore, the alteration in DIAGNOdent value brought about by the staining was unstable, with a return to a new elevated baseline during the monitoring period.

Whilst it was apparent that the introduction of stain into lesions was able to affect their DIAGNOdent analysis, the possibility of an effect brought about by 'removal' of stain was not yet established. To investigate this question, a further group of extracted teeth with pre-cavitation occlusal caries was examined with the DIAGNOdent prior to exposure to a bleaching protocol. Whilst a marked visual change in the lesions was noted following bleaching, analysis of pre and post-bleaching DIAGNOdent values was unable to demonstrate a significant change. Nevertheless, subjective analysis of plotted data for individual specimens indicated an unpredictable effect of exposure to the bleaching protocol on DIAGNOdent analysis.

A further issue concerning the use of the DIAGNOdent device is its ability to detect caries at the margins of amalgam restorations. To investigate this issue, an experiment was devised involving partial caries removal from grossly carious extracted teeth prior to their restoration with dental amalgam. The position of the residual caries at the amelodentinal junction was noted prior to restoration and the ability of both visual inspection and DIAGNOdent analysis to detect this caries was assessed. The ability of the DIAGNOdent device in this respect was not demonstrably superior to visual inspection alone and resulted in sensitivity values of only 40 – 50 percent. Whilst the clinical validity of performance measured in this way is subjective, the figures were comparable to other published work assessing other techniques for detecting residual caries.

The work described above would seem to raise some doubts over the ability of the DIAGNOdent device to detect caries by virtue of detection of demineralisation *per se* and the apparent influence of exogenous stain questions further the nature of the mode of action of the device. Consequently, further experiments were undertaken to investigate more closely the distribution of the fluorescence being measured by the DIAGNOdent device.

Extracted molar teeth with pre-cavitation occlusal caries were assessed both visually and with the DIAGNOdent. Serial 100μm sections were then removed from the surface of the lesions and the DIAGNOdent value recorded at each increment. In this way the DIAGNOdent value was recorded through the full depth of the lesion until

either a return to a below-threshold DIAGNOdent value was reached or the pulp chamber was breached. Although variations in DIAGNOdent value were evident within each lesion, this technique was inconclusive in determining a consistent pattern of fluorescence within the lesion.

Consequently, a further experimental technique was developed to allow longitudinal mapping of the distribution of the fluorescence signal within each lesion. A further group of extracted molar teeth with pre-cavitation occlusal caries was selected, specimens sectioned longitudinally and the cut surface 'mapped' using the DIAGNOdent device. Most specimens exhibited a rise to a peak DIAGNOdent value within the lesion and a gradual decrease to a level below the threshold for clinically significant caries as the analysis moved through the base of the lesion and into the sound dentine beneath. Nevertheless, within this trend, there existed considerable variation and, as with the serial section technique, it was not possible to demonstrate a consistent pattern of fluorescence within these lesions.

Finally, it was attempted to relate the distribution of fluorescence within the lesion measured in this way with the distribution of demineralisation as measured by transverse microradiography. However, practical difficulties were encountered and it proved impossible to establish a profile of the mineralisation using transverse microradiography for the lesions under study.

6.2 Conclusions on Individual Aims of the Research

The objective of the research described in this thesis was to investigate the efficacy of the DIAGNOdent device and the specific aims of the investigations undertaken were listed in section 1.9 as follows.

1. To establish the ability of the DIAGNOdent system to detect demineralisation in artificially created carious lesions.

2. To examine its ability to assess demineralisation quantitatively.

3. To carry out a comparison with another optical caries detection system based on fluorescence (QLF \ clin Optical Measuring System).

4. To investigate the potential for influence from other fluorescent molecules such as exogenous staining materials.

4. To assess the ability of the DIAGNOdent to detect residual caries at the margins of restorations.

6. To examine, in greater detail, the phenomenon of fluorescence from carious lesions by investigating the pattern of fluorescence and relating this to the pattern of mineral loss within the lesion.

In respect of these aims and in light of the completed experimental work, a number of conclusions can be made

1. The DIAGNOdent device is able to detect demineralisation in lesions created artificially by exposure to a demineralising solution. Despite a very small perceived change in DIAGNOdent value, relative to undemineralised controls, a significant difference between control and demineralised groups was demonstrated.

2. The DIAGNOdent device is able to quantify demineralisation in artificially created carious lesions. The DIAGNOdent values of artificially created lesions were shown to correlate significantly with the duration of exposure of the lesions to the demineralising solution. Nevertheless, the degree of correlation was weak.

3. The ability of the QLF\clin system to quantify demineralisation in artificial carious lesions is greater than that of the DIAGNOdent device. For the same lesions, the correlations of all QLF parameters with demineralisation time were greater than the correlation of DIAGNOdent value with demineralisation time.

4. Exogenous staining materials are able to exert a significant effect on the DIAGNOdent value for a carious lesion. This effect is not stable and may be susceptible to change over a given period of time.

5. The DIAGNOdent device is not able to accurately detect residual caries at the margin of an amalgam restoration. Use of the DIAGNOdent for this purpose confers no significant advantage over visual inspection alone.

6. The distribution of fluorescence within a carious lesion, as measured by the DIAGNOdent, is inconsistent. No demonstrable relationship with the distribution of demineralisation exists.

6.3 Null Hypothesis (No.)

On the basis of the experimental work described in this thesis, there is sufficient evidence to reject the Null Hypothesis (No.) that the DIAGNOdent Caries Detection System is unable to detect or quantify demineralisation.

6.4 Overall Conclusion

The fundamental basis for the detection and quantification of carious lesions by the DIAGNOdent is the registration of altered physical characteristics of carious hard tissues relative to the surrounding sound tooth structure. Experimental work has demonstrated the ability of the device to detect and quantify demineralisation. However, a major limitation of the method is that an increased DIAGNOdent reading could represent *any* change in the physical properties of the tooth structure including caries, disturbed tooth development or mineralisation as well as deposits of calculus or organic materials such as exogenous stain. As a result, blind acceptance of the DIAGNOdent value as a true indicator of the carious status of the tooth under study is not appropriate and clinical judgement is a fundamental prerequisite for using the instrument as an aid to the clinical detection of dental caries.

6.5 Further Study

There is clear evidence for the potential of exogenous stain to influence the DIAGNOdent's evaluation of carious lesions *in vitro*. However, additional considerations such as the influence of saliva as well as tongue action, mastication and oral hygiene practices are difficult to estimate and may lead to differences between the observations made in this experimental work and the clinical situation. Simulation of some of these factors is possible and an artificial oral cavity has been described by Shu (1998). Study of the effect of staining on lesions held in conditions more closely resembling the *in vivo* situation is certainly of interest and this is a possibility for future study. Alternatively, an *in situ* trial would allow study of the behaviour of lesions in response to staining within the oral cavity *in vivo*. Wefel (1990) has described the use of intra oral appliances designed to contain an enamel specimen and it may be appropriate to seek ethical approval for a further experiment of this type.

The work described in sections 5.1 and 5.2 failed to demonstrate a correlation between DIAGNOdent value and clinical extent of caries. Neither was there any obvious relationship with the lesion depth as measured by the point at which the DIAGNOdent value fell below threshold. Further investigation into the value of initial DIAGNOdent assessment as a predictor of carious extent would be of value given the desirability of a means of predicting the clinical extent of caries prior to undertaking its restoration. The occlusal lesions studied were of considerable complexity in terms of the general topography of the lesion e.g. spread along the

amelodentinal junction and further work to assess the value of DIAGNOdent assessment in predicting lesion extent in natural smooth surface or even artificially created lesions may offer a better model for study.

Work undertaken here to identify where, within the lesion, the fluorescence detected by the DIAGNOdent was concentrated was inconclusive and the exact nature and distribution of fluorophores remains uncertain. Further experimental work to isolate the fluorescent molecules within the lesion would be of value and personnel with the necessary expertise in this respect have been identified. Given that this work has suggested that the DIAGNOdent, although able to detect caries, may have a relatively poor ability to detect demineralisation, this would progress the understanding of the precise mode of action of the DIAGNOdent in detecting dental caries.

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APPENDIX 1

DIAGNOdent ANALYSIS OF UNSTAINED ARTIFICIAL LESIONS

DIAGNOdent Analysis of Unstained Lesions (Examiner 1)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
0	1	0	1	0	1	0	1	0
0	0	0	0	0	0	0	0	0
0	1	0	1	0	0	0	0	0
0	0	1	0	1	0	0	0	0
0	1	1	1	1	0	0	0	0
0	2	3	2	3	0	0	0	0
0	1	0	1	0	0	0	0	0
0	1	1	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0
0	1	1	1	1	0	0	0	0
8	2	1	0	1	0	0	0	1
8	3	4	0	0	1	0	0	0
8	2	3	2	2	0	0	0	0
8	0	2	2	3	0	0	0	0
8	2	0	1	1	0	0	0	0
8	2	2	2	2	0	0	0	0
8	2	0	0	3	0	0	1	2
8	0	0	2	1	1	1	0	0
8	5	0	-1	1	0	1	0	1
8	0	1	0	0	0	0	0	1
24	1	2	0	1	2	4	0	0
24	1	1	0	1	0	0	0	1
24	1	-1	1	1	0	0	0	0
24	0	0	2	2	2	2	0	1
24	2	3	0	0	1	0	0	0
24	0	0	6	5	1	0	2	1
24	1	1	0	3	1	0	0	0
24	0	0	2	1	1	0	1	1
24	1	2	0	1	1	1	0	0
24	4	3	3	1	0	0	0	0

DIAGNOdent Analysis of Unstained Lesions (Examiner 1 continued)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
48	1	0	1	2	0	1	1	0
48	1	1	1	2	0	0	2	0
48	0	0	3	3	0	1	1	0
48	1	1	6	2	0	0	2	1
48	1	1	-1	-1	0	1	1	1
48	1	2	0	0	0	0	0	0
48	1	2	-1	2	0	0	0	0
48	0	0	1	0	0	0	1	0
48	3	2	7	10	0	0	0	0
48	1	-2	1	1	0	0	-2	-1
72	0	1	1	0	0	0	0	0
72	0	1	1	2	2	3	0	0
72	2	3	0	0	0	0	0	0
72	1	2	4	5	0	0	-1	-1
72	0	0	1	0	0	0	1	0
72	0	1	1	1	1	0	1	0
72	2	0	1	2	0	1	2	2
72	0	0	0	6	0	0	0	0
72	1	2	1	1	0	0	1	1
72	1	1	1	2	0	1	0	1
96	3	-1	0	1	0	0	1	0
96	0	0	3	2	1	0	0	0
96	1	0	0	0	0	0	1	1
96	2	3	0	1	1	0	1	1
96	1	0	2	2	0	0	1	0
96	1	2	2	1	2	2	0	1
96	0	2	1	1	0	0	0	1
96	2	0	1	1	0	0	0	0
96	4	2	1	2	0	0	0	0
96	2	2	0	2	4	3	0	1

DIAGNOdent Analysis of Unstained Lesions (Examiner 2)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
0	1	0	1	0	3	1	3	1
0	0	1	0	1	0	0	0	0
0	1	0	1	0	0	0	0	0
0	0	1	0	1	0	0	0	0
0	1	1	1	1	0	0	0	0
0	2	3	2	3	1	0	1	0
0	1	0	1	0	1	0	1	0
0	1	1	1	1	1	2	1	2
0	0	0	0	0	2	0	2	0
0	1	1	1	1	0	0	0	0
8	2	1	0	1	0	0	1	1
8	2	4	0	0	0	0	0	0
8	1	3	1	2	1	1	0	1
8	0	2	2	3	1	0	1	1
8	2	0	1	0	2	2	0	-1
8	2	2	2	2	1	0	1	2
8	2	0	0	3	1	1	0	1
8	0	0	2	3	3	2	1	0
8	5	0	-1	1	0	-1	-1	0
8	0	1	1	0	-1	0	1	0
24	1	2	0	1	2	3	0	2
24	1	1	0	1	1	0	1	3
24	1	-1	0	1	0	0	1	0
24	0	0	2	2	0	2	0	1
24	2	3	0	0	2	3	1	1
24	0	0	5	5	1	2	1	2
24	1	1	0	3	2	1	0	0
24	1	0	2	1	1	0	3	2
24	1	2	0	1	1	2	3	1
24	3	4	1	3	1	1	4	1

DIAGNOdent Analysis of Unstained Lesions (Examiner 2 Continued)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
48	1	0	1	2	0	0	0	0
48	1	1	1	2	1	1	2	3
48	0	0	5	3	0	1	2	0
48	1	2	6	5	2	2	2	1
48	1	1	-1	0	1	0	2	0
48	1	2	0	0	1	0	1	0
48	1	2	-1	2	1	2	6	3
48	0	0	1	0	1	1	1	2
48	3	2	7	10	0	0	1	0
48	1	-2	1	1	0	1	14	15
72	0	1	1	0	0	1	0	0
72	0	1	1	2	1	2	2	1
72	2	2	0	0	0	0	0	0
72	1	2	4	5	0	0	1	2
72	0	0	1	0	0	1	1	1
72	0	1	1	1	4	2	1	1
72	2	0	1	2	1	3	1	2
72	1	0	2	6	1	0	2	2
72	1	2	1	0	1	0	0	0
72	1	1	1	2	0	2	1	0
96	2	-1	0	1	1	0	2	3
96	0	0	2	2	0	1	0	0
96	1	0	0	0	0	0	0	1
96	2	3	0	1	2	1	16	8
96	1	0	2	2	1	0	2	1
96	1	2	2	1	0	2	1	0
96	0	2	1	1	1	1	2	2
96	2	0	1	1	2	1	2	1
96	4	2	1	2	5	10	0	0
96	2	2	0	2	1	2	1	1

DIAGNOdent Analysis of Unstained Lesions (Examiner 3)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
0	0	0	0	0	0	0	0	0
0	0	1	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	2	0	2	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	1	0	1	0	0	0	0
0	0	1	0	1	1	2	1	2
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
8	1	2	1	0	0	0	0	0
8	5	3	1	1	0	2	0	0
8	0	1	4	2	0	1	0	0
8	0	1	1	0	0	0	0	0
8	0	0	1	2	0	0	1	1
8	1	2	1	2	0	0	1	0
8	0	0	7	5	1	0	0	2
8	0	3	1	1	1	1	1	0
8	0	1	0	0	1	1	-1	1
8	0	0	1	1	0	0	0	0
24	1	2	1	1	2	2	0	0
24	0	-2	1	0	0	0	1	0
24	-1	-1	1	0	0	0	0	0
24	0	0	-1	0	2	3	1	1
24	0	0	0	0	3	4	1	0
24	0	1	4	3	2	2	2	1
24	0	0	0	-1	2	2	1	1
24	0	0	0	1	0	0	0	0
24	1	1	0	0	2	1	5	4
24	1	0	1	1	1	1	0	0

DIAGNOdent Analysis of Unstained Lesions (Examiner 3 Continued)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
48	0	1	0	2	0	0	0	0
48	1	0	0	1	1	1	2	1
48	-1	0	5	3	1	1	1	0
48	0	2	5	6	0	0	5	4
48	0	2	-2	-1	0	0	2	1
48	0	0	1	0	1	1	0	0
48	2	1	2	3	1	2	12	23
48	1	0	2	1	0	1	0	0
48	1	2	2	0	0	0	1	2
48	1	1	1	0	0	0	53	51
72	0	1	0	1	0	0	0	0
72	1	0	1	1	2	2	0	1
72	1	2	2	1	0	0	0	1
72	0	1	4	2	-1	0	2	0
72	0	0	0	1	1	1	1	1
72	0	0	2	3	9	11	2	2
72	0	1	3	3	2	2	3	3
72	1	0	10	13	1	0	6	5
72	2	3	0	-1	1	1	1	1
72	1	2	0	0	0	0	0	0
96	1	1	0	0	1	1	3	3
96	1	1	1	0	0	-2	1	0
96	0	0	1	0	0	0	1	1
96	3	1	0	0	0	2	51	52
96	1	1	1	0	0	0	3	2
96	0	0	4	3	0	0	0	0
96	0	2	1	2	1	1	2	2
96	0	0	2	3	1	0	1	2
96	3	1	0	-1	-3	3	0	0
96	2	1	0	1	5	6	1	1

DIAGNOdent Analysis of Unstained Lesions (Examiner 4)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
0	0	0	0	0	0	1	0	1
0	0	0	0	0	0	0	0	0
0	-1	0	-1	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	1	1	1	1	0	-1	0	-1
0	1	1	1	1	1	0	1	0
0	0	0	0	0	0	0	0	0
0	3	2	3	2	2	0	2	0
0	0	0	0	0	0	0	0	0
0	1	1	1	1	0	0	0	0
8	1	1	1	1	0	1	0	1
8	6	4	0	0	0	1	0	0
8	-2	-1	1	1	1	0	0	0
8	2	3	2	2	0	-1	0	0
8	0	0	-1	1	1	0	1	1
8	2	1	3	2	0	0	1	2
8	0	0	3	3	1	1	0	1
8	0	0	1	1	0	1	0	1
8	0	0	0	0	-1	-1	0	0
8	-1	-2	0	1	1	0	0	1
24	1	0	0	1	2	3	0	0
24	0	1	0	0	0	0	0	0
24	-3	-2	1	1	0	0	0	0
24	1	1	0	0	0	2	0	0
24	2	1	1	1	2	3	1	1
24	0	0	-4	5	1	0	3	1
24	-3	-2	1	1	0	0	0	0
24	-2	-2	1	1	0	0	1	0
24	1	1	1	1	1	4	1	2
24	2	0	2	2	0	0	0	0

DIAGNOdent Analysis of Unstained Lesions (Examiner 4 Continued)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
48	0	0	-2	-3	1	1	0	1
48	1	0	2	1	0	0	4	3
48	1	1	3	4	1	1	0	1
48	1	1	1	1	1	1	1	3
48	0	1	1	1	0	0	2	1
48	2	0	4	3	1	1	0	0
48	2	4	3	4	1	0	1	4
48	3	3	1	2	0	0	2	1
48	1	3	4	9	0	0	1	0
48	1	0	0	-4	1	0	25	25
72	0	0	1	1	1	0	2	0
72	0	-1	1	-2	1	2	4	1
72	3	2	0	0	0	0	0	0
72	2	0	3	2	-1	0	0	2
72	1	0	0	0	0	0	1	0
72	0	-2	3	3	4	4	1	1
72	2	1	2	2	4	2	1	3
72	2	3	5	6	0	0	2	2
72	0	1	1	2	1	0	0	1
72	1	0	2	2	1	1	0	0
96	6	5	-1	0	0	0	4	2
96	2	3	2	1	1	1	0	1
96	1	1	0	0	0	0	1	1
96	1	1	1	0	3	1	1	8
96	1	1	2	2	0	1	2	1
96	0	0	2	2	0	1	0	0
96	1	1	1	0	1	1	2	1
96	1	0	2	1	1	0	1	0
96	1	2	3	3	1	2	0	0
96	2	-1	1	2	0	2	0	0

APPENDIX 2

QLF ANALYSIS OF UNSTAINED ARTIFICIAL LESIONS

QLF Analysis of Unstained Lesions (Examiner 1)

Demin	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
Time (Hrs)	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
0	-11.5	-19	0	-11.5	-19	0	-10	-10	0	-10	-10	0
0	-11.1	-23	0.1	-11.1	-23	0.1	-10.5	-11	0	-10.5	-11	0
0	-11.1	-20	0.2	-11.1	-20	0.2	-10.6	-14	0	-10.6	-14	0
0	-10.2	-11	0	-10.2	-11	0	-10.6	-15	0	-10.6	-15	0
0	-10.9	-22	0	-10.9	-22	0	-10	-10	0	-10	-10	0
0	-11.9	-21	0	-11.9	-21	0	-13.3	-20	0	-13.3	-20	0
0	-11.8	-22	0.4	-11.8	-22	0.4	-10.6	-15	0	-10.6	-15	0
0	-10	-10	0	-10	-10	0	-10.6	-13	0	-10.6	-13	0
0	-10.3	-13	0	-10.3	-13	0	-11.5	-19	0.1	-11.5	-19	0.1
0	-10.9	-15	0	-10.9	-15	0	-11.3	-16	0	-10.8	-16	0
8	-13.6	-25	3.2	-11.4	-20	0.1	-10.5	-14	0	-10.7	-14	0
8	-20.4	-36	6.9	-10.3	-13	0	-14.2	-76	4.7	-12.5	-23	0.2
8	-11.7	-20	0.4	-10.5	-16	0.1	-10.7	-17	0.1	-10.9	-15	0.1
8	-10.8	-17	0.1	-11.1	-17	0.2	-10.3	-12	0	-10.4	-12	0
8	-11.8	-22	0.1	-11.3	-19	0.6	-10	-10	0	-10.7	-17	0.1
8	-11.4	-18	0	-18.5	-65	0.8	-10.9	-15	0.1	-10.5	-14	0.1
8	-13.2	-27	0.7	-13.3	-18	0	-12.6	-22	0.3	-11	-19	0.1
8	-11	-15	0	-11	-17	0.4	-10.3	-11	0	-10.7	-14	0
8	-12.3	-24	0.2	-10	-10	0	-15.4	-27	6.6	-10.5	-13	0
8	-10.9	-19	0	-11.7	-21	0.7	-11	-18	0.2	-10.3	-11	0
24	-11.8	-23	0.2	-13.9	-64	1.5	-11.6	-20	0.1	-10.6	-13	0
24	-14.1	-37	1.6	-12	-25	1	-11	-18	0.2	-12.7	-24	0.7
24	-12	-30	0.4	-11.4	-17	0.3	-10.8	-13	0	-10.9	-17	0
24	-13.6	-27	2.9	-13.6	-29	2.8	-13	-24	3.1	-10.3	-11	0
24	-12.1	-19	0	-13.5	-34	0.8	-12.9	-24	0.4	-11	-17	0.2
24	-12.6	-27	2.8	-20.7	-35	3.7	-17.2	-44	1	-11.5	-23	1
24	-13.7	-27	4.9	-11.7	-27	0.2	-12	-21	0.6	-11	-18	0
24	-11.9	-24	1.8	-12.2	-26	1.4	-12	-24	0.5	-14.7	-34	0.4
24	-13.5	-31	3.6	-11.1	-28	0.6	-17.3	-41	1.6	-12.9	-47	2.9
24	-13.8	-26	2.5	-14.6	-34	4.2	-10.9	-17	0.1	-10.3	-11	0

QLF Analysis of Unstained Lesions (Examiner 1 Continued)

Demin	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
Time (Hrs)	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
48	-13.8	-33	4.1	-14.5	-42	3.9	-17.6	-47	7.8	-10.8	-19	0
48	-13.5	-32	2.4	-14.8	-42	3.5	-12.7	-28	1.5	-12.3	-24	1
48	-21.2	-43	8.9	-17.4	-40	3.3	-16.1	-33	2.3	-18.8	-35	5
48	-16.4	-36	4	-14.6	-26	2.1	-16.4	-37	2.5	-16.9	-33	0.8
48	-17	-29	3.5	-17.6	-27	0.9	-12.9	-33	1	-13.3	-19	0
48	-22.4	-43	7.3	-12.3	-26	0.6	-17	-38	5.9	-10.6	-14	0.1
48	-14.3	-26	3.8	-10	-10	0	-11.7	-26	0.5	-14.2	-30	1.4
48	-20.1	-41	2.4	-13.8	-27	0.7	-14.3	-33	4.7	-10.5	-13	0
48	-16.3	-31	5.3	-24.6	-49	4.3	-13.5	-30	3.5	-14.1	-28	1.7
48	-19.2	-37	6.3	-25.7	-44	3.9	-11.5	-22	0.9	-13	-31	0.2
72	-14.3	-24	2.9	-17.9	-39	4.7	-16.6	-32	3.5	-13.8	-33	1.5
72	-14.6	-34	1.8	-23.7	-43	4.1	-15.4	-31	6.9	-16.9	-43	2.9
72	-14.6	-29	1.4	-13.4	-31	0.7	-11.8	-24	0.2	-18.5	-35	0.3
72	-17.7	-32	4.4	-12.6	-30	0.1	-10.9	-13	0	-15.5	-30	3.6
72	-20.9	-39	6.1	-13.1	-34	0.7	-15.5	-36	4.4	-13.4	-27	1
72	-21.4	-36	2.6	-15.4	-36	2.9	-13.8	-30	3	-20.9	-48	4.7
72	-21.6	-38	3.7	-18.3	-43	2.8	-20.7	-65	5.7	-18.2	-46	2.3
72	-14.8	-36	4.7	-21.8	-46	1.3	-12.5	-28	0.8	-10.6	-17	0.2
72	-16.1	-37	3.6	-20.4	-42	3.5	-11	-15	0	-18.5	-39	5.3
72	-19.3	-34	4.9	-13.7	-27	1.3	-13.3	-33	3.1	-10.8	-13	0
96	-19.7	-52	7.2	-16.3	-40	3.8	-23.8	-44	6.7	-13.8	-37	2.2
96	-24.3	-40	7.5	-13.5	-31	1	-12.9	-24	3.1	-15.6	-42	5.7
96	-18.4	-36	3.3	-18.3	-44	5.4	-15	-35	3.1	-11.5	-22	0.4
96	-24.9	-41	6	-25.2	-50	7.2	-16.5	-32	4.5	-17.4	-36	2.3
96	-17.8	-40	5.1	-14	-34	1.6	-12.5	-31	1.2	-23.3	-45	4.4
96	-25.9	-42	6.1	-22.1	-70	5.1	-10	-10	0	-15.1	-35	1.7
96	-25.6	-54	6.9	-15.4	-38	6.1	-16.8	-32	4.7	-13.7	-27	2.2
96	-29	-48	5.7	-23.4	-52	3.9	-16.4	-42	4.5	-14.1	-32	3.1
96	-21.4	-42	5.9	-13.8	-33	1.6	-13.9	-30	0.9	-21.4	-52	0.8
96	-17.7	-40	4.4	-32.9	-54	4.9	-21.1	-52	5.9	-18.4	-38	3.1

QLF Analysis of Unstained Lesions (Examiner 2)

Demin	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
Time (Hrs)	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
0	-11.6	-18	0.7	-11.6	-18	0.7	-10.9	-16	0.1	-10.9	-16	0.1
0	-11.2	-15	0	-11.2	-15	0	-10.3	-13	0	-10.3	-13	0
0	-10.9	-17	0.4	-10.9	-17	0.4	-10.6	-14	0	-10.6	-14	0
0	-10.3	-12	0	-10.3	-12	0	-10.6	-15	0	-10.6	-15	0
0	-11	-21	0.4	-11	-21	0.4	-10.2	-11	0	-10.2	-11	0
0	-11	-18	0.1	-11	-18	0.1	-11	-17	0.1	-11	-17	0.1
0	-10.9	-14	0	-10.9	-14	0	-10.4	-12	0	-10.4	-12	0
0	-16.2	-31	0.2	-16.2	-31	0.2	-10.8	-15	0.1	-10.8	-15	0.1
0	-11.1	-15	0	-11.1	-15	0	-10.9	-15	0	-10.9	-15	0
0	-10.2	-11	0	-10.2	-11	0	-10	-10	0	-10	-10	0
8	-11.5	-20	0.2	-11.5	-20	0.1	-12.7	-38	1	-10.3	-13	0
8	-17	-38	3.4	-11.2	-18	0.6	-15.6	-52	3.1	-11.5	-19	0.1
8	-11.9	-21	2.7	-10.7	-14	0	-14.7	-32	1.7	-10.6	-14	0
8	-11.6	-21	0.1	-10.6	-14	0	-10.4	-12	0	-10.7	-17	0.1
8	-12.1	-22	0.2	-11.9	-21	1.2	-10	-10	0	-13.2	-18	0
8	-17.9	-35	4.4	-13	-23	0.2	-10.4	-13	0	-10.8	-16	0.1
8	-11.5	-17	0	-13	-38	0.9	-11.7	-20	0.3	-12.3	-22	1.2
8	-10.7	-14	0	-12.3	-24	2.9	-10	-10	0	-11	-18	0.1
8	-11.2	-18	0.1	-11	-20	0.1	-12.7	-32	3	-11.9	-26	0.3
8	-11	-18	0	-12	-21	0.1	-15.2	-37	0.7	-10	-10	0
24	-12.3	-26	0.4	-13.5	-60	2.2	-11.8	-20	0.2	-10.9	-16	0
24	-16.8	-42	2.1	-14.2	-31	1.4	-10.7	-15	0	-12.1	-20	0.2
24	-17.5	-43	5.8	-12.3	-24	1.7	-12	-22	0.8	-10.6	-15	0
24	-13.9	-26	2.7	-12.2	-27	1.3	-12.9	-29	3.1	-10	-10	0
24	-12.2	-18	0	-14.4	-43	3.7	-11.6	-33	0.1	-11	-18	0.1
24	-13.3	-28	3.4	-18	-34	3.2	-16.2	-42	1.1	-12.9	-27	1.6
24	-11.6	-19	0.5	-12.1	-34	1.1	-11.4	-20	0.2	-16.8	-55	1.7
24	-13.4	-26	1.6	-12.8	-36	2	-10.7	-14	0	-18.5	-47	1.4
24	-12.8	-26	2.6	-10.7	-17	0.2	-10.3	-11	0	-12.2	-24	0.5
24	-15.6	-36	3.9	-14.6	-26	2	-10.9	-17	0	-11.3	-22	0.2

QLF Analysis of Unstained Lesions (Examiner 2 Continued)

Demin	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
Time (Hrs)	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
48	-12.9	-30	1.4	-13.6	-34	3.1	-17.1	-39	6.1	-10.4	-13	0
48	-15.8	-30	2.7	-14.6	-38	3.6	-13.5	-33	1.4	-11.3	-20	0.8
48	-21.9	-38	7.3	-17.4	-43	3	-18	-32	4.1	-17.5	-42	5.7
48	-16.9	-48	3.3	-13.9	-28	3	-14.6	-36	1.2	-18.4	-35	0.9
48	-18.1	-30	2.9	-17.1	-29	1.3	-12.7	-30	0.4	-12.5	-25	0.2
48	-21.3	-47	6.3	-12.5	-28	1.1	-14	-31	3.4	-11.1	-15	0
48	-19.3	-34	5.7	-11.7	-19	1.1	-13.7	-27	2.8	-14.6	-28	2.2
48	-18.8	-31	2.6	-14.7	-31	0.9	-14.9	-34	5.6	-11.2	-19	0.4
48	-13.9	-26	3.1	-24.4	-51	4.3	-14.2	-29	3.8	-19.3	-43	4.5
48	-16	-31	4	-23.9	-39	4.3	-14.7	-29	4.3	-12.6	-27	1.9
72	-15.8	-26	3.2	-17.3	-41	3.6	-16	-60	3	-13.4	-30	0.8
72	-11.1	-17	0	-24	-52	2.4	-15.6	-29	6.6	-13.5	-34	0.7
72	-24.8	-40	6.4	-15.9	-42	3	-13.1	-31	1.2	-18.5	-35	0.3
72	-16	-33	3	-12.8	-34	0.7	-10.6	-15	0	-14.8	-31	5
72	-18.5	-37	5.5	-12.5	-29	2	-15.3	-29	3.7	-13.9	-32	2.7
72	-21.9	-40	1.9	-13.6	-26	0.3	-13.6	-36	2.6	-24.4	-49	5.4
72	-21.2	-37	2.7	-18.3	-44	2.5	-24.6	-62	10.5	-20.4	-38	2.8
72	-15.1	-50	2	-17.7	-48	2	-12.6	-30	0.7	-11.9	-42	1.1
72	-17	-35	3.7	-20.8	-42	4.1	-14.7	-29	4.1	-12.4	-24	1.4
72	-21.4	-38	5.8	-12.3	-24	0.6	-12.8	-27	2.7	-19.5	-47	0.5
96	-26.7	-52	6.3	-17.5	-46	3.3	-25.8	-47	5.6	-12.3	-27	1.4
96	-18.1	-34	3.5	-13.5	-30	1	-12.7	-24	0.3	-14	-39	2.3
96	-15.1	-30	4.3	-16.4	-39	3	-14.5	-41	4.2	-11	-18	0
96	-21.8	-40	6.1	-32	-55	8.1	-18.8	-38	7.5	-18.6	-36	1.6
96	-23.6	-44	6.6	-14.8	-35	2.3	-12.4	-30	1.1	-21.4	-41	4.4
96	-28.1	-49	5.3	-20.2	-48	6.2	-11.9	-20	0.8	-14.4	-37	1.3
96	-20.7	-36	6	-16.3	-44	3.3	-20.5	-33	4.4	-19.1	-57	2.1
96	-30.1	-54	5.8	-21.7	-47	6	-27.3	-58	6.6	-13.1	-28	1
96	-26.3	-49	4.4	-14.2	-30	2.2	-15.1	-37	3.3	-10.6	-15	0
96	-20.8	-56	6.6	-33.3	-53	7.2	-23.8	-46	5.5	-18.8	-38	5.5

QLF Analysis of Unstained Lesions (Examiner 3)

Demin	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
Time (Hrs)	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
0	-10.5	-13	0	-10.5	-13	0	-10.7	-14	0	-10.7	-14	0
0	-11	-15	0	-11	-15	0	-10.2	-12	0	-10.2	-12	0
0	-11.1	-17	0.1	-11.1	-17	0.1	-10	-10	0	-10	-10	0
0	-14.6	-33	1.5	-14.6	-33	1.5	-10.6	-15	0	-10.6	-15	0
0	-46.8	-80	4.6	-46.8	-80	4.6	-10.5	-13	0.1	-10.5	-13	0.1
0	-11.7	-25	0	-11.7	-25	0	-10.4	-12	0	-10.4	-12	0
0	-12	-21	0.3	-12	-21	0.3	-10.2	-12	0	-10.2	-12	0
0	-10.6	-13	0	-10.6	-13	0	-20.9	-60	0.6	-20.9	-60	0.6
0	-12	-12	0	-12	-12	0	-10.7	-15	0	-10.7	-15	0
0	-10.3	-12	0	-10.3	-12	0	-12.3	-22	2	-12.3	-22	2
8	-11.1	-16	0.2	-10.8	-16	0.1	-11.1	-17	0.1	-10.3	-11	0
8	-11.5	-19	0.4	-11	-21	0.1	-10.5	-13	0	-11	-12	0
8	-19.5	-14	7.9	-10.5	-57	0	-12.1	-24	0.3	-11.4	-19	0.3
8	-12.4	-19	0.5	-11.1	-31	0.2	-11.3	-20	0.1	-10	-10	0
8	-12.4	-19	0.2	-11.3	-25	0.3	-10.7	-14	0	-10.7	-16	0
8	-11.8	-18	0.3	-10.9	-30	0.4	-10.1	-11	0	-10.9	-16	0.1
8	-14.1	-22	1.2	-12.1	-29	0.4	-15.6	-53	1.6	-12	-22	0.7
8	-17.6	-12	0.7	-10.4	-39	0	-10.5	-12	0	-11.1	-18	0.1
8	-15.4	-12	0.2	-10.4	-30	0	-11.9	-26	1.3	-10.8	-14	0
8	-10.8	-24	0	-12.2	-16	0.2	-11.4	-20	0.2	-10.7	-15	0
24	-11.8	-59	0.1	-13.7	-20	1.6	-12.3	-28	1.2	-10.4	-14	0
24	-14.2	-30	0.4	-14.3	-36	1.5	-12	-23	0.6	-15.8	-38	1
24	-13.5	-27	0.8	-13.6	-28	1.2	-11.2	-22	0.1	-10.8	-12	0
24	-10.7	-25	0.1	-12.8	-15	2.5	-11.6	-20	0.4	-10.2	-11	0
24	-11	-29	0.2	-13.1	-17	2.8	-49	-82	1.1	-11.1	-18	0
24	-12	-24	1.8	-12.9	-27	1.4	-17	-48	1.3	-12.5	-30	1
24	-12.8	-40	0.7	-12.4	-40	1	-12.3	-20	0.2	-12	-24	0.2
24	-12	-30	1.4	-12.7	-23	2.1	-14.2	-29	2.7	-16.6	-45	0.6
24	-12.8	-20	4.2	-11.7	-32	1.6	-13.8	-31	0.7	-12.4	-28	1
24	-13.9	-29	2.9	-14.4	-33	0.9	-10.7	-16	0	-10.8	-18	0

QLF Analysis of Unstained Lesions (Examiner 3 Continued)

Demin	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
Time (Hrs)	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
48	-17.7	-38	4.4	-15	-30	4.1	-13.8	-33	1.2	-11.7	-22	0.5
48	-11.5	-50	0.3	-17.2	-19	5.8	-13.4	-30	1.1	-12.2	-24	0.5
48	-20.5	-55	6.4	-20.1	-40	2.9	-16.5	-33	1.5	-16.6	-31	2.9
48	-17	-27	5.5	-14.2	-43	1.5	-12.6	-30	1	-17.5	-29	0.9
48	-17.5	-35	3.3	-16.6	-34	1.4	-12.7	-24	3.2	-11.4	-23	0.7
48	-16.3	-25	4.5	-12.3	-37	0.6	-12.7	-25	2.1	-11.2	-21	0.1
48	-17.1	-21	3.9	-11.3	-34	0.4	-12.5	-28	1.2	-13.3	-29	1.3
48	-18	-32	2.3	-13.4	-31	1.4	-14	-33	2.5	-10.2	-12	0
48	-17.2	-49	6.4	-24.8	-32	4.4	-13.5	-27	0.9	-17.4	-37	1.5
48	-19.5	-47	4	-25.2	-35	5.2	-12.9	-24	1.3	-13	-27	0.6
72	-15.7	-46	2.5	-18.8	-32	3.3	-15.8	-42	3	-13.8	-35	1.3
72	-12	-52	1.1	-21.6	-22	2.1	-16.5	-36	6.6	-17.2	-38	2.4
72	-12.8	-39	1.1	-14.5	-29	1.2	-13.4	-38	0.6	-10.5	-13	0
72	-14.3	-28	3.3	-15.6	-31	3.3	-10.9	-17	0.1	-14.7	-29	4.4
72	-23.1	-41	6	-13.8	-36	3	-12.7	-25	0.7	-13.6	-28	1.1
72	-23.1	-32	2.3	-13.3	-43	1.1	-13	-31	1.2	-22.1	-49	5.4
72	-24.1	-42	2.6	-19	-44	2.2	-15.7	-41	2.5	-18.8	-37	2.6
72	-19.2	-55	7.2	-19.2	-45	3.4	-12.1	-22	0.4	-12.6	-22	2.1
72	-15	-45	3.3	-21.7	-35	3.9	-12.1	-24	0.3	-12	-24	0.9
72	-22	-33	6.6	-13.7	-39	2	-12.9	-29	0.7	-20.9	-60	0.6
96	-21.8	-41	4.2	-18	-51	6.7	-21.5	-43	6.3	-18.4	-38	1.1
96	-28.1	-29	4.6	-14	-46	0.7	-17	-33	2.2	-13.8	-32	1.6
96	-19.2	-38	7.6	-16.6	-35	4.3	-11.5	-21	0.3	-11.6	-23	0.4
96	-14	-47	3.9	-27.6	-34	6.8	-16.1	-31	5.7	-20.5	-42	1.2
96	-22	-34	5.9	-14.5	-40	2.7	-12.9	-24	1	-12.7	-26	0.9
96	-18.4	-52	6.3	-21.4	-42	5.5	-10.8	-17	0	-13.3	-24	1.1
96	-20.6	-43	4.4	-16.5	-49	3.7	-18.1	-33	5.2	-13	-25	0.3
96	-27.6	-53	5.8	-23	-47	6.5	-18.6	-49	3.1	-12.8	-24	0.4
96	-25.4	-33	4.2	-14.1	-43	1.5	-15.7	-31	2.5	-10.9	-16	0.1
96	-18.5	-53	4	-33.7	-45	6	-23.4	-44	7.8	-17.8	-37	3.3

QLF Analysis of Unstained Lesions (Examiner 4)

Demin	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
Time (Hrs)	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
0	-10.3	-11	0	-10.3	-11	0	-29.4	-56	3.4	-29.4	-56	3.4
0	-10.8	-22	0	-10.8	-22	0	-11.8	-24	0.1	-11.8	-24	0.1
0	-10.7	-24	0	-10.7	-24	0	-16.4	-40	2	-16.4	-40	2
0	-11.5	-17	0	-11.5	-17	0	-12.7	-25	0.5	-12.7	-25	0.5
0	-18.1	-46	0.2	-18.1	-46	0.2	-14.3	-32	0.9	-14.3	-32	0.9
0	-12.2	-22	0.3	-12.2	-22	0.3	-11	-14	0	-11	-14	0
0	-12	-21	0.1	-12	-21	0.1	-12.4	-30	0.5	-12.4	-30	0.5
0	-10.6	-14	0.1	-10.6	-14	0.1	-33	-78	1.6	-33	-78	1.6
0	-12.9	-19	0	-12.9	-19	0	-12	-26	0.2	-12	-26	0.2
0	-11	-12	0	-11	-12	0	-10.8	-15	0	-10.8	-15	0
8	-11.4	-18	0.3	-10.8	-14	0	-10.6	-13	0	-12.6	-26	0.4
8	-11.6	-22	0.1	-10.3	-14	0	-21.1	-50	2.4	-11.2	-17	0.1
8	-11.2	-16	0	-10.3	-12	0	-10.7	-14	0	-30.1	-61	5.2
8	-11.5	-19	0	-11.1	-16	0	-11.3	-18	0	-11.9	-27	0.3
8	-12.5	-19	0	-12	-27	0.9	-14.6	-33	1.8	-12.4	-28	0.2
8	-11.7	-19	0	-12.4	-18	0.1	-13.6	-35	1.6	-25	-70	3.5
8	-11.5	-19	0.1	-10.6	-16	0.1	-19.2	-52	2.4	-16.5	-37	0.2
8	-11.9	-22	0.1	-10.5	-13	0	-35	-63	4.8	-23	-50	2.9
8	-11.6	-20	0.3	-10.2	-11	0	-21.7	-56	6.6	-12.5	-26	0.3
8	-11.3	-23	0.3	-12	-23	0.1	-43.2	-73	5.5	-10.6	-13	0
24	-11.2	-19	0.3	-14	-63	1.2	-11.4	-16	0	-11.8	-17	0
24	-13	-24	0.1	-11.4	-19	0.3	-18.7	-46	5.3	-11.4	-19	0.1
24	-11.8	-25	0.1	-13.1	-26	1.3	-22.1	-42	1.9	-13.4	-28	0.8
24	-12.6	-26	1.8	-11.4	-20	0.4	-29.8	-64	2.9	-24.7	-50	2.2
24	-11	-19	0.1	-13.1	-26	0.3	-21.7	-62	2.4	-15.1	-38	2
24	-11.9	-21	0.2	-13.1	-25	1.5	-18.1	-44	1.2	-17.2	-55	0.9
24	-11.9	-27	0.9	-11.9	-25	0.4	-14.3	-29	2.2	-16.2	-47	1.7
24	-12.4	-37	1.6	-12.4	-23	0.3	-16.9	-47	1.2	-11.2	-20	0.1
24	-13.9	-38	1.3	-12	-36	0.6	-24.8	-64	1.9	-18	-43	2.9
24	-17.1	-45	2.4	-17.2	-33	1.4	-13.2	-30	0.2	-12.6	-27	0.5

QLF Analysis of Unstained Lesions (Examiner 4 Continued)

Demin	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
Time (Hrs)	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
48	-15.1	-32	3.1	-14.9	-44	2.3	-10.7	-15	0	-35.2	-84	2.1
48	-12.1	-23	0.8	-15.6	-45	1.8	-11.1	-18	0.1	-18.2	-44	2.3
48	-16.7	-42	4.5	-17.9	-40	3.2	-11.9	-19	0	-14.5	-30	0.1
48	-15.2	-38	3.3	-13.9	-23	1.2	-17.4	-48	2.3	-34	-62	5.5
48	-17.4	-30	2.4	-14.5	-29	0.8	-25.7	-58	1.2	-27.7	-71	2.3
48	-12.3	-34	0.4	-12.8	-28	1.1	-22.4	-59	2.5	-27.2	-64	5.7
48	-14.9	-30	3	-11.1	-17	0	-12.4	-29	1.7	-13.4	-29	1.6
48	-20.1	-35	2.2	-14.2	-31	0.4	-10.8	-14	0	-10.8	-16	0
48	-19.1	-42	4.4	-21.8	-46	3.2	-21.2	-53	3.1	-11.9	-20	0.1
48	-17.7	-39	2.4	-24.9	-44	3.6	-18.9	-41	1.5	-26.1	-58	1.5
72	-15.2	-31	2.1	-17.9	-49	2.2	-17	-49	2	-14.3	-43	0.6
72	-12.4	-24	0.6	-17.3	-44	2	-12.4	-28	0.3	-16.3	-67	2.2
72	-17.4	-42	3	-14.5	-37	0.7	-11.8	-24	0.3	-20	-54	2.3
72	-13.5	-25	1	-12.7	-34	0.4	-14.7	-42	1.3	-12.1	-24	0.1
72	-17.6	-39	4.8	-13.5	-31	0.4	-19.9	-46	1.3	-12.3	-23	1.2
72	-24.2	-47	2.1	-13.5	-31	0.1	-15.5	-32	0.2	-21.2	-51	3.8
72	-21.9	-47	2.1	-20.2	-51	2.4	-16.6	-42	0.6	-13.2	-32	1.1
72	-13.9	-34	2.5	-19.4	-45	1	-22.8	-47	1.8	-11.7	-25	0.3
72	-15.4	-35	2.3	-20.8	-51	5	-17.4	-55	3	-12	-27	0.1
72	-19.7	-40	5.1	-14.1	-32	2.2	-18.8	-51	2.8	-21.5	-62	1.1
96	-19	-47	3.2	-15.8	-41	2.8	-11.1	-17	0	-13.1	-32	1
96	-14.1	-30	1.8	-13.4	-27	0.4	-14.4	-45	0.9	-21.5	-60	3.8
96	-14.3	-33	1.9	-17.4	-40	1.9	-12.6	-25	2.3	-12.5	-26	0.1
96	-23.9	-39	5.7	-26.9	-47	4.7	-12.3	-27	0.6	-11.4	-19	0.2
96	-15.6	-37	1.5	-15.7	-42	2	-17	-49	2	-12.1	-25	0.1
96	-28.3	-48	5	-21.3	-51	1.9	-32.9	-71	2.5	-38.9	-68	4.1
96	-14.4	-35	0.9	-16.2	-45	2.1	-33.3	-82	2.3	-14.8	-31	1.8
96	-19.1	-47	5.3	-20.7	-47	4.8	-34.2	-64	4.4	-17.6	-53	0.8
96	-25.6	-50	3.1	-14.4	-32	2.6	-12.3	-25	0.1	-15.9	-37	0.7
96	-14	-30	1.7	-23.2	-44	4.8	-23.8	-53	2.9	-35.7	-60	3.5

APPENDIX 3

DIAGNOdent ANALYSIS OF STAINED ARTIFICAL LESIONS (A PILOT STUDY)

DIAGNOdent Values of 96 hour Carbopol Lesions Before and After Exposure to Different Staining Protocols.

Specimen No.	Staining Time (Hours)	Pre-stain DIAGNOdent Value	Post-stain DIAGNOdent Value
1	6	1	2
2	6	0	3
3	6	1	1
4	6	2	2
5	6	1	1
6	6	1	-1
7	6	0	2
8	6	2	2
9	12	3	4
10	12	2	2
11	12	-1	0
12	12	0	2
13	12	0	0
14	12	2	2
15	12	0	1
16	12	2	2
17	24	2	15
18	24	0	19
19	24	2	6
20	24	2	13
21	24	0	18
22	24	3	74
23	24	0	9
24	24	0	21
25	48	2	43
26	48	2	99
27	48	1	71
28	48	1	33
29	48	1	37
30	48	0	9
31	48	2	11
32	48	2	59
33	72	0	10
34	72	1	20
35	72	2	41
36	72	1	99
37	72	1	87
38	72	1	34
39	72	2	26
40	72	2	10

APPENDIX 4

DIAGNOdent ANALYSIS OF STAINED ARTIFICIAL LESIONS

DIAGNOdent Analysis of Stained Lesions (Examiner 1)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
0	1	2	1	2	1	1	1	1
0	0	0	0	0	0	0	0	0
0	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	1	1	1	1	0	0	0	0
0	0	1	0	1	0	1	0	1
0	0	0	0	0	1	0	1	0
0	0	0	0	0	2	1	2	1
0	1	0	1	0	1	0	1	0
0	1	0	1	0	1	0	1	0
8	2	1	2	2	1	2	3	2
8	4	5	1	3	0	1	0	1
8	13	9	6	5	1	2	1	0
8	3	2	2	2	1	1	1	0
8	5	3	13	8	0	0	2	2
8	7	8	10	8	1	2	0	0
8	5	6	7	4	2	1	1	2
8	3	4	4	5	1	2	1	1
8	1	5	6	6	0	1	1	3
8	2	4	10	10	0	2	0	0
24	14	12	18	14	3	2	0	1
24	4	5	6	5	0	0	5	6
24	11	8	6	6	0	0	1	1
24	6	8	5	7	8	9	0	1
24	5	5	4	3	2	1	1	1
24	4	6	12	15	2	3	17	15
24	5	10	12	7	1	2	12	10
24	12	9	13	12	0	1	10	8
24	16	17	3	3	10	9	1	2
24	14	14	14	14	1	5	1	2

DIAGNOdent Analysis of Stained Lesions (Examiner 1 Continued)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
48	17	25	13	15	5	8	6	2
48	2	5	8	5	1	1	2	3
48	12	12	20	15	0	0	3	2
48	42	39	4	3	10	12	10	4
48	12	13	6	4	1	1	1	1
48	17	19	19	9	17	15	0	0
48	21	20	14	18	0	1	8	10
48	10	12	12	17	10	10	2	2
48	15	23	14	16	2	2	2	2
48	18	22	21	24	3	2	10	11
72	30	28	6	7	7	6	0	0
72	12	10	13	10	5	3	1	1
72	20	20	7	9	11	10	1	0
72	20	19	5	5	1	2	3	2
72	31	31	4	11	2	1	0	0
72	71	47	15	26	10	5	10	11
72	15	15	13	10	25	14	2	2
72	5	7	29	34	0	0	1	2
72	23	17	15	14	3	2	1	1
72	16	16	7	6	0	1	2	2
96	11	18	5	4	16	9	13	13
96	63	75	5	4	3	2	0	0
96	10	9	20	25	1	0	8	6
96	21	22	24	16	4	6	11	7
96	41	43	10	5	2	4	2	4
96	99	99	6	6	1	3	0	0
96	98	70	15	16	11	14	12	15
96	34	33	19	12	10	12	2	3
96	28	38	6	4	13	12	3	6
96	10	9	13	12	16	16	2	3

DIAGNOdent Analysis of Stained Lesions (Examiner 2)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
0	1	0	1	0	0	1	0	1
0	1	0	1	0	0	0	0	0
0	0	0	0	0	0	1	0	1
0	0	1	0	1	0	0	0	0
0	1	0	1	0	0	0	0	0
0	1	1	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	1	0
0	1	1	1	1	1	1	1	1
0	1	0	1	0	1	0	1	0
8	2	2	3	5	1	1	2	2
8	7	5	1	3	1	2	1	1
8	7	7	6	7	0	2	1	1
8	3	2	2	3	1	0	2	1
8	4	5	9	10	2	1	3	2
8	11	9	5	3	1	1	0	1
8	6	4	3	7	1	1	3	2
8	4	3	4	8	1	1	0	1
8	4	4	6	6	0	0	2	2
8	4	4	10	11	0	1	1	0
24	14	10	17	17	2	1	0	0
24	1	0	3	5	1	0	7	6
24	8	6	6	10	0	2	1	0
24	7	8	4	5	10	7	1	0
24	3	4	4	6	1	1	2	1
24	4	4	18	18	2	3	13	11
24	7	6	9	14	4	2	11	8
24	10	9	8	9	0	0	7	12
24	16	15	3	3	8	4	1	1
24	12	10	18	10	2	3	1	2

DIAGNOdent Analysis of Stained Lesions (Examiner 2 Continued)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
48	30	31	13	16	5	4	4	3
48	4	5	6	4	2	0	2	1
48	12	12	18	18	2	1	2	2
48	37	33	3	3	14	13	5	11
48	13	10	9	13	2	0	0	2
48	20	16	8	5	13	12	1	0
48	22	22	16	16	0	0	9	11
48	14	12	20	15	11	13	2	1
48	12	20	17	17	1	2	3	2
48	23	21	27	27	2	1	12	9
72	29	31	3	4	6	5	1	0
72	11	13	11	10	4	2	1	2
72	29	37	11	11	12	8	2	2
72	22	20	5	4	2	3	2	2
72	23	30	4	10	1	3	2	1
72	42	45	23	17	8	7	8	13
72	22	25	12	14	19	16	1	0
72	6	10	31	20	1	0	3	2
72	23	17	12	14	1	2	1	1
72	15	16	7	7	2	1	3	2
96	19	19	3	5	14	12	15	10
96	92	70	1	4	1	3	3	2
96	10	10	21	26	1	1	9	7
96	23	23	18	17	4	8	14	9
96	47	46	7	5	1	3	2	3
96	99	99	9	9	2	3	1	2
96	99	69	9	6	10	8	10	13
96	33	21	14	15	13	12	3	4
96	38	47	3	6	13	11	3	2
96	9	10	12	16	12	14	0	1

DIAGNOdent Analysis of Stained Lesions (Examiner 3)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
0	0	0	0	0	-1	0	-1	0
0	0	1	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	44	46	44	46	0	1	0	1
0	0	1	0	1	0	0	0	0
0	0	1	0	1	1	2	1	2
0	0	0	0	0	0	1	0	1
0	0	2	0	2	1	0	1	0
0	1	0	1	0	1	0	1	0
8	6	6	2	4	0	1	0	1
8	3	4	4	2	1	0	0	0
8	6	4	4	5	0	0	0	0
8	3	5	4	3	0	1	0	0
8	7	7	1	2	0	0	0	0
8	2	3	4	3	0	1	0	0
8	6	6	2	2	1	0	1	2
8	2	3	3	4	0	0	0	0
8	4	3	4	3	1	0	0	0
8	6	6	9	6	0	1	0	0
24	7	8	7	5	1	0	3	0
24	2	3	1	2	0	0	0	1
24	3	2	2	3	0	0	0	0
24	6	4	3	1	1	1	0	1
24	4	4	2	3	2	3	0	1
24	7	6	5	4	3	1	0	-2
24	4	2	4	3	1	0	0	-1
24	2	3	2	4	1	0	3	4
24	7	5	1	1	1	2	8	3
24	8	11	7	7	0	1	0	0

DIAGNOdent Analysis of Stained Lesions (Examiner 3 Continued)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
48	6	7	7	8	1	1	0	1
48	0	0	2	4	0	0	0	1
48	9	13	10	8	1	2	3	2
48	8	7	2	2	1	0	1	2
48	4	5	5	4	0	0	0	0
48	14	9	3	4	3	1	0	0
48	12	11	4	9	4	5	5	5
48	3	2	4	2	4	4	0	0
48	4	5	9	11	2	1	3	2
48	9	11	8	10	3	1	6	4
72	7	12	3	2	1	0	0	0
72	2	1	2	0	1	1	2	1
72	7	4	4	3	1	0	1	0
72	9	10	3	4	-1	0	0	0
72	11	12	8	8	0	0	0	1
72	15	21	8	11	0	1	2	2
72	13	13	7	6	1	2	1	1
72	7	7	9	9	0	0	2	3
72	7	5	7	10	0	1	3	1
72	8	7	1	1	0	0	0	0
96	7	8	1	2	4	4	0	0
96	14	11	3	2	1	1	2	1
96	8	8	8	8	0	0	0	1
96	3	5	6	5	1	2	0	0
96	34	20	4	7	1	0	2	2
96	37	39	4	3	0	1	0	0
96	36	27	8	9	4	5	3	4
96	5	4	5	5	4	4	1	1
96	7	4	7	8	1	2	0	0
96	6	7	8	5	0	0	3	1

DIAGNOdent Analysis of Stained Lesions (Examiner 4)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	1
0	0	1	0	1	0	0	0	0
0	6	5	6	5	0	1	0	1
0	0	1	0	1	1	0	1	0
0	0	0	0	0	2	1	2	1
0	0	1	0	1	1	2	1	2
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
8	2	0	7	8	2	2	0	0
8	4	5	4	2	1	1	0	0
8	6	4	2	3	0	0	1	0
8	1	0	2	3	0	0	1	0
8	3	2	7	2	0	0	1	1
8	2	1	6	6	0	1	1	1
8	0	0	3	1	1	0	2	1
8	4	3	2	4	3	2	0	0
8	0	1	2	3	0	1	0	0
8	1	1	3	3	2	2	2	0
24	6	6	4	3	3	3	1	0
24	2	1	2	6	0	0	4	1
24	0	0	3	3	0	0	0	0
24	1	0	4	4	1	1	0	1
24	2	3	3	1	0	0	0	0
24	7	5	3	7	3	4	1	1
24	2	2	4	4	0	0	0	0
24	7	3	4	3	1	1	2	3
24	4	4	2	3	4	4	4	2
24	2	5	6	5	0	1	4	5

DIAGNOdent Analysis of Stained Lesions (Examiner 4 Continued)

	Lesion Group							
	Abraded		Abraded		Unabraded		Unabraded	
Demin	'Carbopol'		'Glasgow'		'Carbopol'		'Glasgow'	
Time (Hrs)	I	II	I	II	I	II	I	II
48	9	14	12	9	0	1	1	0
48	1	2	4	3	1	0	4	2
48	11	8	4	6	1	1	0	0
48	6	10	1	0	4	3	2	2
48	3	2	5	5	0	0	0	1
48	10	4	3	4	1	1	0	0
48	1	1	19	14	1	1	6	5
48	4	5	3	2	2	1	0	0
48	1	2	9	7	1	1	1	1
48	6	5	7	5	3	4	4	3
72	1	2	3	5	1	1	1	0
72	0	0	4	3	2	2	0	0
72	8	7	4	2	1	3	0	1
72	2	4	7	7	0	2	1	2
72	19	18	7	7	1	1	2	3
72	12	10	6	6	1	1	3	3
72	1	0	7	7	3	4	1	1
72	3	2	13	14	0	0	0	0
72	5	4	0	0	1	1	0	1
72	6	10	3	3	1	1	0	0
96	7	4	6	10	4	5	3	1
96	3	6	8	8	1	0	1	1
96	5	7	13	10	0	0	1	2
96	5	5	10	6	0	0	1	1
96	10	9	4	3	0	1	1	0
96	32	40	4	3	2	1	0	1
96	7	12	4	3	1	3	3	4
96	7	7	4	3	2	1	2	2
96	8	8	5	4	2	1	1	0
96	0	4	6	5	5	3	1	0

APPENDIX 5

QLF ANALYSIS OF STAINED ARTIFICAL LESIONS

QLF Analysis of Stained Lesions (Examiner 1)

Demin Time (Hrs)	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
0	-10.4	-16	0	-10.4	-16	0	-10.7	-15	0.1	-10.7	-15	0.1
0	-10.7	-15	0.1	-10.7	-15	0.1	-18.2	-38	0.2	-18.2	-38	0.2
0	-11.5	-19	0.2	-11.5	-19	0.2	-10.8	-18	0	-10.8	-18	0
0	-11.2	-21	0.1	-11.2	-21	0.1	-10.4	-13	0	-10.4	-13	0
0	-11.1	-17	0	-11.1	-17	0	-10.8	-17	0	-10.8	-17	0
0	-11.8	-22	0.2	-11.8	-22	0.2	-10.8	-16	0	-10.8	-16	0
0	-11.5	-19	0	-11.5	-19	0	-11.3	-19	0.1	-11.3	-19	0.1
0	-10.6	-15	0.1	-10.6	-15	0.1	-16.9	-36	0.6	-16.9	-36	0.6
0	-10.8	-15	0	-10.8	-15	0	-10.8	-15	0	-10.8	-15	0
0	-10.9	-19	0	-10.9	-19	0	-11.1	-14	0	-11.1	-14	0
8	-13.4	-28	2	-14.3	-35	1.9	-14.8	-43	3.2	-10.6	-14	0
8	-20.7	-78	6.1	-12.2	-24	0.3	-14.3	-45	0.7	-13.4	-29	0.3
8	-13.1	-45	3.6	-11.3	-18	0.2	-14.9	-41	2.1	-10.8	-17	0.1
8	-13.3	-30	3	-14.5	-37	2	-12.5	-22	0	-10.3	-12	0
8	-12.9	-31	1.7	-21.3	-52	5.6	-11.7	-18	0	-13.5	-39	0.6
8	-12.2	-29	1.1	-17.9	-55	4.3	-11.3	-21	0.5	-10.5	-14	0
8	-14	-29	5.6	-13.7	-31	2.5	-17.1	-43	0.8	-12.5	-28	0.4
8	-12.1	-29	0.6	-15.7	-43	3.6	-11	-16	0	-11.1	-19	0.1
8	-14.6	-50	2.2	-14.2	-34	1.2	-13.1	-33	2.2	-11	-16	0
8	-13.3	-41	1.3	-17.3	-47	3.9	-16.9	-37	7.9	-10.6	-13	0
24	-15.4	-37	3	-33.9	-71	6.5	-23.3	-52	5.6	-10.5	-14	0
24	-17.9	-43	2.2	-34.1	-65	3.1	-12.2	-25	1.1	-19.5	-46	0.3
24	-20.9	-48	4	-28.4	-64	3.5	-11.4	-28	0.3	-22.3	-71	0.5
24	-25	-44	4.7	-23.9	-49	4.2	-25.7	-52	4	-10.9	-17	0
24	-13.8	-34	1.8	-34.2	-66	8.3	-11.6	-17	0	-11.5	-20	0.1
24	-19.5	-48	3.4	-35	-66	3.3	-21.5	-60	3	-20	-43	4.8
24	-19	-38	8.7	-25.6	-62	3.7	-20.1	-40	1.4	-20.1	-57	3.1
24	-18.8	-46	7.2	-26.1	-70	5.5	-16.1	-41	3.7	-22	-67	1.7
24	-20.5	-50	5	-17.7	-35	3.9	-18.8	-67	3.2	-16	-44	2.3
24	-28.1	-52	3.6	-31.3	-71	6.1	-20	-55	0.8	-11.9	-20	0.1

QLF Analysis of Stained Lesions (Examiner 1 Continued)

Demin Time (Hrs)	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
48	-25.6	-48	5.5	-37.4	-74	5.8	-17.9	-44	6.7	-10.8	-19	0
48	-20.1	-44	3.3	-37.9	-72	6.5	-14.5	-35	2	-14.4	-38	0.3
48	-33.7	-64	8.7	-31.8	-75	6.8	-24	-47	3.3	-20.8	-49	3.9
48	-28.3	-56	5.8	-23.8	-55	2.2	-21.5	-53	2.6	-25.4	-57	1.9
48	-30.2	-49	3	-21.6	-62	3.2	-17.8	-44	3.6	-16.5	-36	0.3
48	-24.7	-58	5.3	-27.4	-67	4.2	-25.9	-54	8.3	-10.6	-14	0
48	-25.9	-58	4.1	-13.5	-33	2.1	-11.5	-22	0.5	-18.8	-37	2.4
48	-30.4	-53	3	-31.1	-71	4.2	-30.9	-67	8	-10.4	-13	0
48	-27.9	-64	6.7	-46.1	-79	5.8	-29.6	-59	6.2	-29.7	-65	3.4
48	-31.6	-63	4.8	-55.5	-82	5.9	-19.3	-45	1.9	-32.8	-69	7.2
72	-29.9	-51	3.6	-36.2	-69	4.9	-27.5	-57	3.1	-15.5	-39	3.5
72	-18.5	-43	5.5	-48	-80	4.9	-20.3	-57	6.2	-16	-51	1.9
72	-34.3	-59	7.2	-28.6	-64	8.7	-26.1	-74	4.5	-10.4	-12	0
72	-27.9	-49	5	-20	-62	5.1	-12	-20	0.2	-18.1	-46	4.6
72	-34.7	-61	7.4	-22.1	-62	6	-19.2	-56	4.1	-16.9	-48	3.2
72	-30.9	-63	6	-30.3	-64	5.8	-18.6	-52	4.5	-33.8	-68	5.6
72	-29.6	-56	4.5	-34.6	-71	3.5	-38.5	-75	7	-22.4	-50	2.9
72	-26.2	-55	8.6	-38.4	-75	7.4	-15.5	-36	0.8	-15.3	-30	2.6
72	-24	-61	5	-50	-81	8	-22.7	-59	4.6	-13	-26	1.3
72	-36.5	-63	8	-41.2	-69	5.4	-19.2	-56	4	-10.9	-15	0.1
96	-33.9	-70	7.4	-43.8	-77	5.6	-37	-75	6.6	-13.7	-36	1.3
96	-37.4	-61	5.8	-26.3	-66	4.5	-34	-66	4.5	-16.8	-60	4.7
96	-32.1	-61	5.1	-35.6	-72	7.1	-18.3	-48	3	-11.5	-20	0.2
96	-34.2	-60	7.2	-61.4	-84	8	-24.9	-54	6.7	-25.3	-54	3.5
96	-33.3	-64	7.1	-30.7	-69	5	-18.7	-47	2.6	-26.8	-50	5.8
96	-24.3	-55	4	-32.5	-83	9.1	-26.4	-58	4.9	-16.7	-46	1.2
96	-30.6	-65	10	-31.9	-72	6.4	-38.3	-70	6.8	-15.2	-39	1.5
96	-30.4	-62	8.2	-53.7	-79	8.7	-49.7	-76	12	-13.2	-28	0.3
96	-28.6	-61	4.1	-34.7	-72	4	-29.2	-57	4.6	-11.2	-17	0.1
96	-35.8	-63	7.4	-59.1	-84	5.6	-48.5	-78	6.2	-19.2	-44	4.5

QLF Analysis of Stained Lesions (Examiner 2)

Demin Time (Hrs)	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
0	-10.7	-15	0	-10.7	-15	0	-10.6	-14	0	-10.6	-14	0
0	-10.7	-15	0	-10.7	-15	0	-10.9	-19	0	-10.9	-19	0
0	-10.5	-13	0	-10.5	-13	0	-10.3	-11	0	-10.3	-11	0
0	-10.7	-14	0	-10.7	-14	0	-10.6	-13	0	-10.6	-13	0
0	-10.6	-14	0	-10.6	-14	0	-11.1	-23	0	-11.1	-23	0
0	-10.9	-14	0	-10.9	-14	0	-12.7	-27	0.2	-12.7	-27	0.2
0	-14.7	-39	0.5	-14.7	-39	0.5	-11.6	-22	0.1	-11.6	-22	0.1
0	-10.4	-12	0	-10.4	-12	0	-14.7	-36	1.2	-14.7	-36	1.2
0	-11.9	-14	0	-11.9	-14	0	-11.4	-25	0.5	-11.4	-25	0.5
0	-11.5	-16	0	-11.5	-16	0	-10.3	-12	0	-10.3	-12	0
8	-12.9	-29	1.7	-15.6	-36	2.7	-12.4	-25	0.2	-10.8	-14	0
8	-18.8	-78	4.6	-14.8	-41	2.6	-12.8	-32	0.4	-12.7	-28	0.2
8	-15.3	-59	3.9	-11	-17	0.1	-11.4	-27	0.6	-11.8	-21	0.3
8	-13.8	-35	1.7	-14.2	-40	2.5	-12.1	-25	1.8	-10.4	-15	0
8	-14.5	-31	4.2	-20.2	-47	4.6	-10.3	-12	0	-13.7	-34	0.4
8	-13.1	-32	4.5	-18.1	-69	4.9	-11.4	-21	0.2	-11.3	-22	0.2
8	-12.2	-23	1.4	-12.7	-39	1.6	-19.8	-49	0.6	-15	-53	1.7
8	-12.2	-24	0.5	-17.1	-56	4.4	-11.7	-22	0.1	-10.7	-14	0
8	-13.9	-46	1.2	-14.1	-35	3.5	-11.6	-20	0.2	-11	-17	0.1
8	-14.8	-34	2.5	-17.1	-42	3.7	-11.6	-21	0.1	-10.8	-14	0
24	-17.8	-45	2.8	-34.1	-74	5.2	-25.5	-58	7.6	-11	-19	0.2
24	-17.7	-48	2.5	-33.8	-60	3.9	-11.6	-21	1.2	-39.2	-77	1.9
24	-26.9	-51	4	-33.7	-71	5.2	-11.1	-18	0	-19.9	-65	0.3
24	-23.6	-42	4.2	-25.8	-55	7.8	-23	-46	4.1	-11.1	-16	0
24	-13.3	-32	1.1	-30.8	-60	7.5	-11.9	-21	0.1	-12	-23	0.3
24	-15.8	-40	5.3	-33.5	-61	3.6	-23.6	-65	5.1	-16.8	-39	2.7
24	-18.5	-42	6.1	-26.8	-58	3.2	-20.2	-42	1.9	-11	-17	0
24	-19.2	-41	5.4	-25.6	-67	5.8	-13.9	-40	2	-22.4	-67	1.9
24	-23.2	-54	4.9	-16.8	-37	7.8	-18.1	-66	2.8	-21.2	-66	6
24	-26.4	-54	3.1	-29.2	-71	7.1	-11.2	-33	0.1	-10.8	-16	0.1

QLF Analysis of Stained Lesions (Examiner 2 Continued)

Demin Time (Hrs)	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
48	-22.9	-46	3.5	-33.8	-72	7.8	-15.8	-39	2.4	-10.7	-17	0
48	-19.5	-42	2.3	-41.2	-75	5.4	-14.2	-40	1.2	-14.4	-39	0.6
48	-35.6	-68	7.6	-37.2	-78	6.1	-21.9	-45	3.6	-21	-45	3.6
48	-30.3	-55	6.4	-20.9	-41	4.6	-21.5	-56	4.8	-25.2	-53	0.8
48	-27.2	-55	2.6	-19.5	-55	3.1	-18.2	-45	3.8	-17.1	-44	0.3
48	-30.6	-67	10.7	-14.7	-45	2.3	-30.4	-67	5.7	-10.6	-17	0.1
48	-33	-61	7.5	-32.4	-63	10.9	-14.4	-33	1.8	-19.1	-41	1.8
48	-33	-54	3.1	-38.7	-74	5.8	-32.1	-62	10.2	-11.2	-14	0
48	-32.4	-58	6.1	-46.2	-78	4.6	-27.9	-56	5.7	-31.3	-66	3
48	-23.9	-50	7.9	-56.6	-81	8.1	-18.9	-41	1.8	-24.4	-57	4.1
72	-16.3	-46	0	-38.3	-71	4	-28.4	-60	5.8	-15	-41	3.6
72	-18.8	-43	5	-51.6	-82	5.9	-23.6	-66	7.3	-17	-50	3.2
72	-25.1	-48	6.1	-30.7	-65	6.1	-24.8	-42	4.2	-10.6	-18	0.1
72	-28.9	-47	5	-21.1	-65	5.3	-11.8	-20	0.1	-20.1	-46	5.7
72	-34.7	-60	8.1	-25.3	-63	4.9	-22.2	-56	3.8	-17.2	-55	2.1
72	-31.2	-59	6.8	-34.8	-68	8.1	-21.6	-59	4.5	-40.7	-76	7
72	-33	-60	4.1	-47.3	-79	4.5	-37.7	-77	12.7	-23.5	-55	2.5
72	-23.9	-52	7.5	-37.3	-74	6.6	-14.8	-32	0.8	-14.1	-35	1.4
72	-24.4	-61	4.6	-41.6	-78	10.9	-21	-63	2.9	-13	-22	1.2
72	-35.2	-61	6.5	-39.7	-71	4.7	-20.2	-48	6.7	-12.5	-25	0.1
96	-31.2	-68	5.9	-42.8	-78	6	-39.7	-76	7.1	-12.5	-31	1
96	-35.3	-59	5.7	-25.2	-65	4.8	-31.9	-63	4.1	-16.5	-52	1.2
96	-32	-62	6.3	-39.5	-76	7.9	-27.2	-68	4.2	-11.2	-26	0
96	-37.5	-64	8	-62.4	-84	9.4	-25.4	-60	6.3	-26.9	-59	2.2
96	-23.4	-56	6.7	-31.1	-70	6.3	-21.3	-56	5.6	-27.8	-53	5.5
96	-37.5	-65	6.4	-29.4	-75	10	-25	-58	3.7	-16	-40	0.9
96	-30.8	-65	4.9	-35.6	-77	7	-45.6	-73	9.7	-16.6	-46	1.3
96	-30.7	-59	5.8	-53.7	-82	7.1	-46.7	-75	5.6	-15.4	-42	1.5
96	-39.7	-68	7.4	-36.7	-72	3.1	-27.4	-59	4.5	-32.5	-79	1.5
96	-32.1	-61	8.3	-59.2	-86	7	-44.2	-79	9.2	-20.1	-42	3.9

QLF Analysis of Stained Lesions (Examiner 3)

Demin Time (Hrs)	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
0	-11	-12	0	-11	-12	0	-10.9	-16	0.1	-10.9	-16	0.1
0	-10.9	-19	0	-10.9	-19	0	-10.2	-12	0	-10.2	-12	0
0	-12.7	-22	0.9	-12.7	-22	0.9	-11.1	-17	0.2	-11.1	-17	0.2
0	-11.2	-15	0	-11.2	-15	0	-10.8	-14	0	-10.8	-14	0
0	-41.6	-77	3.4	-41.6	-77	3.4	-12	-27	0.1	-12	-27	0.1
0	-11.2	-16	0	-11.2	-16	0	-14.9	-36	1.8	-14.9	-36	1.8
0	-13.1	-30	0.2	-13.1	-30	0.2	-15.9	-31	0.4	-15.9	-31	0.4
0	-10.5	-15	0.1	-10.5	-15	0.1	-11.2	-18	0.1	-11.2	-18	0.1
0	-11.3	-16	0	-11.3	-16	0	-10.9	-16	0	-10.9	-16	0
0	-10.8	-18	0	-10.8	-18	0	-26.8	-56	3.8	-26.8	-56	3.8
8	-11.7	-21	0.5	-13.8	-37	2	-11.5	-19	0.1	-11.1	-19	0.1
8	-17.4	-69	2.7	-12.6	-33	0.6	-12.4	-28	0.9	-10.8	-16	0
8	-12	-26	0.8	-12.8	-29	1.3	-12.3	-32	0.8	-21	-45	2.4
8	-15	-32	2.9	-13.8	-44	3.1	-11.6	-26	0.7	-22.3	-50	5
8	-13.7	-30	1.5	-21	-49	4.5	-11	-19	0	-17.4	-42	4.1
8	-11.8	-48	0.7	-14.4	-62	2.9	-11.3	-19	0	-17.1	-48	1.8
8	-12.1	-23	1.2	-11.3	-19	0.5	-14.4	-46	1.8	-22	-66	1.4
8	-11.7	-27	0.1	-14.7	-34	4.2	-10.9	-23	0	-10.9	-14	0
8	-16.7	-41	0.3	-10.9	-17	0.1	-11.1	-16	0	-40.3	-68	5.6
8	-12.4	-33	1.1	-17.7	-44	4.6	-42.5	-73	10.9	-21.1	-54	5.8
24	-14.3	-39	2.7	-29.3	-69	4.7	-19.4	-48	5.3	-10.8	-17	0.1
24	-14.4	-37	1.5	-29.9	-59	4.7	-11.5	-23	0.3	-17	-43	1
24	-16.6	-41	3.8	-30.7	-64	3.3	-11.6	-22	0.6	-10.8	-15	0
24	-17.2	-41	1.5	-18.7	-44	3.7	-19.2	-39	4.5	-10.9	-17	0
24	-13.4	-29	1.2	-14.6	-31	2	-41.6	-79	1.6	-11	-17	0.1
24	-15.1	-38	4.3	-30	-62	3.5	-17.4	-49	2.4	-16	-52	2.6
24	-13.1	-33	1.7	-16.6	-45	3.3	-18.8	-50	1.8	-17	-47	2
24	-14.3	-29	3.4	-20.5	-54	4.6	-11.8	-25	0.8	-10.9	-18	0.1
24	-16.4	-41	5.6	-14.8	-34	3.8	-17.1	-44	0.4	-28.7	-62	3.2
24	-17.6	-44	3.2	-20.1	-59	6.4	-11.3	-19	0	-12.9	-29	0.3

QLF Analysis of Stained Lesions (Examiner 3 Continued)

Demin Time (Hrs)	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
48	-14.6	-40	3.2	-27.9	-68	6	-15.8	-37	4.9	-11	-18	0.1
48	-17.2	-46	3.6	-25.4	-65	7.8	-15.3	-39	1	-19.5	-50	2.9
48	-28.8	-61	6.1	-26.5	-70	4	-10.9	-16	0	-18.5	-46	2.5
48	-19.8	-50	5.5	-20.9	-45	3.4	-23.1	-53	4.1	-10.7	-14	0
48	-17.1	-39	3.4	-17.6	-39	0.9	-10.7	-14	0	-11.4	-20	0.3
48	-23.5	-62	7.7	-17	-55	3.9	-33.6	-71	6.8	-19.2	-54	0.2
48	-24.1	-57	5.1	-30.1	-63	9.3	-17.6	-40	3.5	-20.6	-59	0.7
48	-28.4	-54	4.7	-28.9	-68	5.8	-12.3	-27	1.2	-27.9	-58	3.6
48	-15	-40	4.7	-42.3	-78	6.9	-18.3	-52	2.9	-17.3	-37	3.6
48	-14.1	-44	1.7	-48.2	-75	7.5	-10	-10	0	-19	-46	1.9
72	-27.1	-46	3.9	-20.9	-54	6.5	-26.8	-60	3.9	-14.4	-39	1
72	-13.3	-35	1.8	-45.9	-83	6	-20.3	-65	5.3	-17.4	-49	0.7
72	-26.1	-55	6.3	-24.5	-61	9.3	-17.1	-39	1.7	-10.8	-20	0.1
72	-24.2	-56	3.9	-17.3	-59	2.7	-11.2	-16	0	-14.8	-43	0.2
72	-17.4	-46	6.6	-19.9	-56	5.9	-12.4	-24	0.6	-23.9	-50	6.3
72	-26.7	-61	5.3	-18.6	-55	4.9	-18.6	-56	1.1	-11.8	-23	0.6
72	-15	-39	3.5	-29	-69	3.1	-15.7	-34	0.6	-15.4	-40	1.9
72	-22.1	-52	8.6	-23.7	-73	4.8	-15.9	-44	1.4	-13.8	-29	1.6
72	-19.3	-47	5.1	-37.8	-76	7.7	-11.8	-25	0.1	-25.9	-59	8
72	-22.9	-56	5.8	-25.6	-50	4.8	-11.5	-19	0.1	-33.5	-65	5.4
96	-25.5	-62	4.9	-26.6	-62	6	-24.1	-64	5.3	-13.1	-37	0.5
96	-23.6	-65	4.7	-15.1	-44	3.1	-27	-60	3.2	-17.6	-53	1.4
96	-17.4	-52	5.4	-20.8	-55	7.7	-20.1	-50	2.8	-11.7	-21	0.4
96	-14	-41	4.5	-56.4	-81	8.3	-12.1	-33	0.2	-23.5	-55	2
96	-16.4	-50	4.2	-26.2	-72	5.5	-15.2	-37	0.8	-20.8	-56	2.3
96	-18.7	-49	6.8	-17.5	-62	3.8	-18.3	-48	3.8	-27.4	-56	1.1
96	-14.7	-45	2.9	-30.1	-70	5.6	-18.7	-52	4.7	-11.1	-17	0
96	-14.3	-39	5.2	-46.8	-77	7	-20.3	-54	3.5	-13.7	-40	1.3
96	-20.1	-50	5.2	-25.6	-65	4.6	-11.8	-23	0.5	-24.3	-60	5.2
96	-22.9	-49	6.1	-51.5	-79	8.3	-20.1	-39	2.3	-11.9	-27	0.2

QLF Analysis of Stained Lesions (Examiner 4)

Demin Time (Hrs)	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
0	-10.9	-15	0	-10.9	-15	0	-11.9	-20	0	-11.9	-20	0
0	-14.2	-34	0.1	-14.2	-34	0.1	-11.8	-17	0	-11.8	-17	0
0	-10.9	-17	0.1	-10.9	-17	0.1	-10.8	-14	0	-10.8	-14	0
0	-11.9	-28	0.1	-11.9	-28	0.1	-10.2	-11	0	-10.2	-11	0
0	-39.2	-72	2.9	-39.2	-72	2.9	-11.5	-21	0.4	-11.5	-21	0.4
0	-11	-15	0	-11	-15	0	-12.2	-22	0.1	-12.2	-22	0.1
0	-14	-30	0.1	-14	-30	0.1	-11	-18	0	-11	-18	0
0	-11.3	-16	0	-11.3	-16	0	-13.7	-22	0	-13.7	-22	0
0	-12.6	-27	0.3	-12.6	-27	0.3	-12.1	-14	0	-12.1	-15	0
0	-10.9	-18	0.1	-10.9	-18	0.1	-13.9	-30	1.4	-13.9	-30	1.4
8	-12.8	-27	1.1	-15.3	-38	2.7	-11.5	-19	0.2	-11.1	-18	0.1
8	-13.3	-36	0.8	-12.5	-30	0.7	-11	-18	0	-11.2	-22	0.1
8	-12.6	-27	1.1	-12.8	-28	1	-11	-17	0	-16.9	-35	2.3
8	-15.3	-67	2.4	-13.6	-33	1.2	-13.3	-30	1.3	-19.7	-38	4
8	-14.6	-35	1.5	-20.9	-54	3.5	-10.8	-20	0.1	-16	-33	3.6
8	-12.7	-31	2.2	-16	-56	2.5	-10.3	-12	0	-12.3	-26	0.4
8	-12.5	-25	0.4	-12.1	-23	0.5	-16.7	-40	2.4	-18.3	-44	0.9
8	-11.6	-22	0.3	-13.1	-31	1.1	-11.1	-18	0.2	-10.8	-17	0
8	-13.8	-59	1.4	-13.4	-34	1.3	-11.8	-41	0.7	-15.6	-35	3.2
8	-13.6	-30	0.7	-15.6	-42	1.8	-18.4	-40	3.4	-12.6	-30	1
24	-13.2	-33	1.5	-22.1	-61	3.7	-11.6	-24	0.1	-10.4	-12	0
24	-13.3	-30	1.4	-27.3	-54	2.3	-11.5	-20	0.3	-11.9	-23	0.3
24	-13.4	-33	0.8	-27.1	-61	3.9	-11.3	-19	0.3	-11	-15	0.1
24	-16.2	-36	0.8	-19.8	-41	2.9	-13.1	-24	1.7	-10.5	-12	0
24	-12	-22	0.5	-13.7	-34	0.9	-25.2	-63	1	-10.7	-14	0.1
24	-18.1	-44	2.8	-27	-51	2.5	-16.3	-48	2	-14.7	-40	3.2
24	-14.9	-33	3.2	-21.9	-61	2.7	-12.9	-25	0.1	-13.6	-29	1.4
24	-16.6	-37	3.8	-19.1	-45	3.9	-10.5	-14	0	-12.6	-21	0.1
24	-13.6	-32	2.2	-18.7	-39	3.3	-11.6	-18	0.1	-15.3	-35	1.4
24	-19.3	-48	2	-20.6	-53	5.1	-11	-16	0	-10.8	-16	0

QLF Analysis of Stained Lesions (Examiner 4 Continued)

Demin Time (Hrs)	Abraded 'Carbopol'			Abraded 'Glasgow'			Unabraded 'Carbopol'			Unabraded 'Glasgow'		
	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area	ΔL_{mean}	ΔL_{max}	Lesion Area
48	-14.9	-42	2.9	-33.1	-62	3.6	-12.4	-25	0.4	-11.2	-19	0.1
48	-13.7	-34	0.8	-29	-59	5	-13.3	-30	0.8	-10.2	-11	0
48	-18	-48	4.3	-21.6	-60	2.3	-11.8	-19	0.1	-12.3	-29	1.2
48	-21.7	-61	3.6	-20.9	-47	1.8	-17.5	-38	3.8	-10.9	-16	0
48	-22.2	-48	2.4	-17.6	-39	0.5	-10.5	-11	0	-11.3	-26	0.3
48	-30.6	-61	4	-27.5	-65	3.9	-11.3	-22	0.1	-22.6	-44	0.4
48	-26	-58	3.1	-21.8	-52	5.4	-11.9	-21	0.8	-13.8	-30	0.6
48	-32	-55	3.3	-25.4	-66	4.3	-11.8	-25	0.8	-10	-10	0
48	-15.7	-43	2.2	-37.6	-76	5.2	-14	-35	1.1	-17.8	-41	2.6
48	-21.4	-51	3.8	-51.6	-79	4.2	-10	-10	0	-14.1	-29	1.6
72	-20.2	-40	2.8	-38.4	-74	4.5	-13.3	-28	0.5	-13.6	-29	1.5
72	-12.4	-27	0.5	-38.7	-75	4.5	-15.7	-32	3.8	-17.1	-39	1.6
72	-19	-52	6.8	-15.6	-46	2	-13.1	-27	1.4	-11.9	-20	0.3
72	-24.8	-44	3.8	-14	-45	2.4	-11.3	-23	0.1	-12.3	-26	0.2
72	-17.4	-44	3.2	-19.2	-50	3.8	-12.4	-24	1.2	-12.3	-24	0.4
72	-20.4	-52	3.6	-28.9	-73	6.2	-15.4	-36	0.9	-13	-25	2.3
72	-16	-40	2.4	-30.6	-65	3.3	-10.5	-14	0	-13.5	-26	0.9
72	-13.7	-33	2.1	-23.8	-69	4.3	-19.5	-38	1.8	-11.6	-22	0.5
72	-18.8	-48	2.8	-34	-76	7.2	-12.2	-22	0.1	-13.5	-30	1
72	-19.3	-46	5.2	-27.9	-59	5.3	-10.8	-16	0.1	-25	-55	3.9
96	-21.3	-54	3.8	-40	-80	5	-19	-42	3.7	-11.8	-22	0.6
96	-16.2	-60	2.5	-23.7	-65	4	-13.6	-28	2	-10.8	-16	0
96	-28.6	-53	3.8	-23.5	-63	5.4	-11.7	-21	0.1	-12	-26	0.1
96	-15.4	-41	4.9	-52.4	-76	5.7	-11.1	-20	0	-12.6	-23	0.7
96	-18.7	-55	3.1	-32.9	-63	3.3	-14.9	-31	0.8	-14.5	-37	1.6
96	-20.4	-48	3.6	-20.5	-67	7.6	-12.7	-29	0.6	-15.6	-28	0.8
96	-17.5	-41	4.7	-34.1	-70	5	-15.3	-31	3.8	-12.6	-28	0.3
96	-21.5	-51	4.8	-38.3	-75	7.4	-12.9	-31	0.7	-10.2	-11	0
96	-19.9	-55	3.8	-20.1	-54	2.8	-11.2	-22	0.2	-13.2	-37	1.1
96	-18	-44	3.4	-58.9	-82	6.8	-18.1	-37	2.1	-11.6	-20	0

APPENDIX 6

DIAGNOdent AND QLF ANALYSIS OF ARTIFICIAL CARIOUS LESIONS EXPOSED TO TEA STAINING PROTOCOL

QLF Analysis of Artificial Lesions at Various Times in relation to Staining

	Lesion Area (mm^2)				ΔL_{max} (%)				ΔL_{mean} (%)			
	time in relation to staining period											
Sepcimen No.	pre-stain	6 days	20 days	27 days	pre-stain	6 days	20 days	27 days	pre-stain	6 days	20 days	27 days
1	4.7	7.4	6.1	7.8	-50	-80	-74	-76	-34	-53.1	-47.6	-52.8
2	6.1	8.3	6.8	8.6	-67	-79	-78	-79	-42.3	-53.4	-49.5	-54.4
3	4.5	6.4	5.4	7.9	-52	-74	-73	-73	-21.3	-43.9	-42	-45.1
4	4.9	8.8	7	7	-50	-81	-82	-80	-32.3	-57.1	-58.1	-58.4
5	4.3	5.9	6.1	6.7	-44	-81	-84	-81	-27.3	-50.9	-54.2	-55.4
6	3.9	4.6	4.6	7.6	-51	-77	-73	-75	-28.3	-51	-47.9	-50.5
7	4.2	9	6.8	5.6	-48	-78	-77	-70	-23.8	-44.6	-44.1	-40
8	3.6	6.2	6.2	4.4	-56	-76	-76	-74	-33.1	-46.5	-46.7	-44
9	3.4	6.4	7	6.2	-35	-75	-80	-74	-17.1	-47.6	-52.6	-46.6
10	6.1	8.3	7.5	7.8	-52	-77	-80	-72	-24.9	-48.8	-46.6	-42.9
11	3.4	7.1	7.1	8.1	-39	-76	-76	-78	-17.5	-46.7	-46.5	-42.2
12	7.5	7	6.7	6.9	-53	-78	-79	-89	-35.6	-55.1	-53.7	-51.6
13	7.7	8.5	7.4	8.4	-43	-79	-80	-75	-24.7	-48.3	-50.3	-40.4
14	3.9	4.8	3.6	5.2	-35	-69	-72	-62	-16.8	-44.4	-42.5	-34.7
15	3.5	3.3	2.8	4.9	-52	-73	-79	-76	-31.9	-48.6	-50	-50.1
16	5.1	5.4	4.8	5.5	-46	-74	-77	-73	-27.1	-45.2	-48.8	-42
17	4.6	6.4	7.8	6	-69	-76	-83	-74	-27.3	-47.8	-50.3	-44
18	5	7	6	5.2	-51	-73	-75	-64	-29.4	-39.9	-41.8	-31.8
19	6.1	7.3	6.1	6.3	-56	-77	-79	-80	-36	-50.4	-52	-44.7
20	3.6	4.9	5.4	4.4	-58	-78	-83	-79	-32.4	-39.1	-46.6	-43.3

DIAGNOdent Analysis of Artificial Lesions at Various Times in relation to Staining

			Peak DIAGNOdent Value									
			Elapsed Time Post-Staining (Days)									
Specimen No.	Pre-stain	Immediately Post-stain	1	2	3	4	5	6	7	8	20	27
1	2	34	15	15	11	14	11	9	10	12	9	12
2	1	35	20	16	12	16	14	8	7	6	15	4
3	2	61	25	30	20	23	17	14	11	16	16	11
4	2	33	20	15	15	14	8	10	10	7	7	7
5	1	22	33	29	32	24	24	21	25	19	21	22
6	1	19	19	12	13	16	9	10	6	5	6	5
7	1	18	13	17	9	6	6	7	6	9	14	9
8	1	11	6	6	5	6	3	3	6	4	5	6
9	1	18	9	14	18	7	9	7	7	8	8	8
10	2	22	9	8	8	6	5	4	4	4	7	8
11	2	15	12	15	13	10	9	11	8	9	10	8
12	2	16	12	11	9	6	4	4	6	5	5	7
13	1	21	12	19	14	12	6	12	7	6	5	5
14	0	12	8	10	8	4	7	8	5	5	6	5
15	1	26	16	13	13	9	12	5	6	6	6	10
16	1	25	26	26	21	20	18	18	18	23	14	10
17	3	23	11	18	11	7	7	4	4	7	5	11
18	3	14	5	6	5	4	4	3	2	3	4	3
19	0	86	92	73	68	72	60	42	41	40	40	35
20	4	97	80	76	40	50	31	20	14	21	20	35

APPENDIX 7

DIAGNOdent ANALYSIS OF NATURAL CARIOUS LESIONS EXPOSED TO TEA AND PEROXIDE

DIAGNOdent Analysis of Peroxide Exposed Natural Occlusal Lesions

	Peak DIAGNOdent Value									
	Pre-Stain Analysis					Post-Stain Analysis				
Specimen No.	1	2	3	4	5	1	2	3	4	5
1	22	16	14	15	13	13	9	8	10	14
3	23	18	14	15	13	16	10	9	11	9
7	29	31	34	38	25	32	18	15	14	11
8	49	33	25	41	41	66	28	36	23	36
10	49	52	38	38	37	51	32	51	21	35
12	39	36	33	39	48	38	58	43	39	53
13	34	38	30	26	28	61	48	53	41	33
16	5	5	3	3	2	2	0	0	1	0
18	37	33	22	14	18	19	22	16	22	20
21	16	20	15	26	17	2	2	1	0	1
24	19	23	24	15	23	26	17	18	13	16
25	23	23	22	22	27	16	8	12	13	10
28	18	23	18	22	21	38	41	40	38	49
29	36	40	27	32	29	46	16	10	15	10
30	3	5	3	5	5	1	0	1	0	4
31	34	32	25	20	24	99	60	75	38	75
33	48	58	58	52	54	17	13	12	11	12
39	12	15	14	15	18	15	13	10	12	7
42	38	41	38	30	27	42	42	37	41	30
43	80	93	62	63	70	99	99	99	99	99
44	41	39	33	30	37	69	55	59	64	44
45	19	16	17	18	22	6	3	3	6	5
48	10	12	11	10	11	12	4	4	4	3
49	64	66	99	74	85	30	18	24	21	11
50	28	34	29	24	31	24	24	24	22	15

DIAGNOdent Analysis of Tea Exposed Natural Occlusal Lesions

	Peak DIAGNOdent Value									
	Pre-Stain Analysis					Post-Stain Analysis				
Specimen No.	1	2	3	4	5	1	2	3	4	5
2	37	64	46	34	49	99	37	43	48	44
4	47	52	36	38	41	51	51	45	42	46
5	94	99	99	97	99	61	99	99	99	99
6	46	39	26	30	27	57	50	30	40	35
9	19	15	12	14	12	36	31	26	22	22
11	12	10	9	7	11	34	7	14	13	13
14	33	35	28	23	34	31	32	34	30	27
15	10	16	11	9	13	34	11	14	12	18
17	12	22	14	15	15	39	29	30	21	27
19	37	15	18	15	12	25	33	23	12	14
20	13	17	23	18	18	67	24	39	32	33
22	20	17	18	19	18	59	42	35	38	23
23	53	44	28	30	24	99	40	69	21	44
26	12	16	12	16	17	39	19	21	18	17
27	34	37	38	32	38	99	50	40	45	59
32	49	60	75	37	67	69	48	45	62	47
34	37	37	27	31	27	34	27	30	33	35
35	6	6	6	6	10	39	17	25	11	15
36	26	20	28	7	29	18	17	19	22	13
37	6	6	6	5	5	34	18	16	19	14
38	19	27	23	27	31	80	39	39	45	37
40	17	20	19	15	16	31	19	22	16	18
41	13	16	12	11	13	17	14	12	14	12
46	1	2	1	1	2	28	9	7	9	4
47	10	11	13	4	10	66	30	26	17	30

APPENDIX 8

VISUAL AND DIAGNOdent ASSESSMENT OF CARIES AT RESTORATION MARGINS

Location of Residual Caries by Visual and DIAGNOdent Assessment

		Examiner 1		Examiner 2	
Specimen Number	True Position of Caries	Visual Examination	DIAGNOdent Examination	Visual Examination	DIAGNOdent Examination
1	DP	DP	DP	DP	DP
2	MP	-	D	-	D
3	DP	-	DP	-	DP
4	M	M	D	-	-
5	MB	-	D	-	D
6	DB	-	-	-	-
7	M	-	-	-	-
8	MP	MP	MP	MP	MP
9	MP	-	-	-	-
10	DP	-	-	-	DP
11	P	-	-	P	-
12	B	-	B	-	B
13	DB	-	-	-	-
14	M	-	DB	-	-
15	MB	MB	MB	MB	MB
16	D	-	-	-	D
17	DB	DB	DB	-	DB
18	DP	-	DP	-	DP
19	D	-	-	-	-
20	MP	-	-	-	M

APPENDIX 9

DIAGNOdent ANALYSIS OF NATURAL CARIOUS LESIONS: TRANSVERSE LESION DISSECTION

DIAGNOdent Values within Lesions (Transverse Section) Specimens 1-10

	Specimen Number									
Depth (microns)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Initial Reading	72	21	33	10	30	11	12	26	50	27
Base of fissure	28	27	33	11	38	13	16	27	39	25
100	10	28	34	11	54	13	19	23	50	23
200	8	27	40	13	58	9	15	31	51	28
300	7	25	55	13	60	9	12	23	52	28
400	8	23	53	14	63	10	8	21	55	29
500	9	27	60	17	69	14	10	51	61	30
600	12	26	89	-	76	14	11	50	74	20
700	12	27	99	-	66	15	10	48	99	18
800	16	34	99	-	71	17	8	51	84	17
900	18	37	99	-	77	18	7	35	73	17
1000	19	43	99	-	84	16	7	21	62	11
1100	-	48	91	-	86	15	7	18	30	20
1200	-	43	83	-	69	19	7	10	29	-
1300	-	34	99	-	35	21	8	7	31	-
1400	-	30	83	-	38	20	6	5	30	-
1500	-	20	66	-	55	17	6	5	25	51
1600	-	26	41	-	59	19	6	3	15	33
1700	35	16	29	22	99	16	6	3	9	40
1800	36	4	26	27	46	12	4	2	5	53
1900	42	0	18	25	36	10	4	3	4	53
2000	30		12	37	27	7	4	2	2	51
2100	29		9	37	22	4				48
2200	28		6	39	17	2				45
2300	23		5	40	15					43
2400	20		4	41	12					40
2500	18		4	43	13					36
2600	15		4	46						
2700	15		5	67						
2800	16		7	54						
2900	16		7	54						
3000	23		7	27						
3100				16						

DIAGNOdent Values within Lesions (Transverse Section) Specimens 11-20

	Specimen Number									
Depth (microns)	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Initial Reading	27	28	22	16	20	17	22	2	21	17
Base of fissure	34	28	27	20	11	20	28	29	29	30
100	34	28	28	25	10	20	32	31	31	31
200	41	29	28	58	10	20	34	30	25	28
300	26	29	28	62	10	24	34	26	25	28
400	24	-	27	36	10	21	44	25	27	39
500	22	-	26	29	10	20	45	22	27	32
600	28	-	38	26	6	26	48	32	30	30
700	19	-	41	20	8	31	48	36	34	40
800	17	-	47	18	13	32	43	36	53	70
900	14	-	51	11	15	-	27	39	99	78
1000	16	-	57	8	15	-	22	44	99	96
1100	14	-	57	7	16	-	21	56	99	99
1200	14	-	59	7	19	14	13	69	99	99
1300	10	-	63	7	20	-	13	83	99	99
1400	17	-	73	9	22	-	11	76	53	57
1500	13	-	-	14	28	29	10	74	41	43
1600	17	22	-	9	38	33	12	71	12	13
1700	20	27	-	7	46	34	10	74	12	4
1800	32	28	70	5	27	24	6	73	12	4
1900	36	33	81	3	29	-	6	75	10	3
2000	54	35	93	3	29	-	2	41	12	
2100	40	34	99		16	16	2	36	11	
2200	19	28	73		4	19	-	25	9	
2300	6	20	57		3	18	-	17	7	
2400	1	26	40		3	26	-	10	7	
2500	1	30	31			20	-	3	5	
2600	1	27	24			20	-		3	
2700		12	17			21	-			
2800		9	11			22	-			
2900		3	8			14	-			
3000			5			15	-			
3100						14	-			
3200						11	-			
3300						10	-			
3400						8	8			
3500						3	3			
3600						2	2			

APPENDIX 10

DIAGNOdent ANALYSIS OF NATURAL CARIOUS LESIONS: LONGITUDINAL LESION DISSECTION

DIAGNOdent Values within Lesions (Longitudinal Section) Specimens 1-12

	Specimen Number											
Depth (µm)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0	7	25	6	7	10	6	14	14	80	31	52	34
100	8	26	6	7	13	9	16	15	93	32	74	34
200	9	23	6	7	14	11	20	19	99	50	99	34
300	12	22	7	9	15	17	23	27	99	66	99	32
400	16	22	7	12	17	22	25	36	99	73	99	29
500	19	24	9	15	20	27	30	45	99	78	99	23
600	22	25	9	17	23	33	34	48	99	68	89	20
700	24	25	10	18	27	39	31	45	99	53	78	19
800	25	26	10	17	32	45	27	40	99	49	74	20
900	23	28	10	16	36	42	26	39	73	46	71	22
1000	21	31	10	15	45	42	30	50	48	45	68	21
1100	18	34	10	13	51	41	35	44	31	43	61	17
1200	15	37	11	13	58	34	40	48	18	41	52	14
1300	13	34	14	13	60	30	41	50	8	44	40	11
1400	11	30	22	16	53	28	46	52	4	50	29	9
1500	9	27	33	17	44	26	55	46	2	55	22	8
1600	7	23	42	14	36	26	63	46	0	58	15	7
1700	7	20	48	13	29	28	76	50		56	11	6
1800	5	19	46	13	21	28	92	45		46	9	4
1900	5	21	36	13	14	27	99	41		38	8	3
2000	4	27	26	13	9	26	97	41		30	6	2
2100	4	33	18	12	6	27	85	41		23	5	2
2200	4	40	12	12	3	25	75	37		20	5	
2300	4	46	9	10	2	24	66	26		17	5	
2400		46	7	10	1	25	57	17		16		
2500		42	7	8	1	25	49	14		15		
2600		35	6	7	0	25	41	14		15		
2700		29	6	6	1	24	34	14		15		
2800		24	7	5	0	23	26	13		14		
2900		20	6	4		20	20	9		13		
3000		18	6	3		18	15	6		12		
3100		16	7	3		16	12	5		11		
3200		14		3		14	10	4		11		
3300		13				12	9	3		10		
3400		11				10	7	2		9		
3500		10				8	6	2		9		
3600		9				7	5			9		
3700		7				6	5			10		
3800		6				5	4			9		
3900		6				4	4			9		
4000		5				3	3			9		
4100		6					4			6		
4200		6										
4300		5										
4400		5										

DIAGNOdent Values within Lesions (Longitudinal Section) Specimens 13-24

	Specimen Number											
Depth (µm)	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
0	1	7	7	4	2	16	13	23	36	26	25	27
100	1	7	7	3	4	17	12	25	42	29	27	31
200	1	6	7	1	6	20	13	20	52	35	33	38
300	1	5	8	1	8	23	13	14	55	41	35	47
400	1	5	9	0	11	27	13	12	57	50	33	56
500	0	5	11	0	13	30	15	12	61	62	31	64
600	0	6	13	0	12	29	18	12	66	69	30	72
700	0	4	14	0	10	28	18	11	68	74	32	82
800	0	5	16		7	26	17	10	64	91	34	93
900	0	6	17		5	20	16	9	59	99	38	99
1000	0	6	18		5	17	13	7	56	99	40	99
1100	0	6	19		5	17	8	6	59	99	42	99
1200	0	5	22		4	16	7	4	60	99	44	99
1300	0	4	26		3	15	5	3	59	99	47	99
1400	0	3	30		3	15	5		56	99	47	99
1500	0	4	33		2	14	5		55	99	44	99
1600		3	35			14	3		54	99	38	99
1700		3	36			15	2		54	97	31	99
1800		4	35			15	2		53	86	23	89
1900		5	31			15			52	78	16	75
2000		6	30			16			49	72	10	65
2100		4	30			15			44	66	6	52
2200		3	33			14			37	60	4	37
2300		1	37			12			36	51	3	24
2400		1	41			10			24	41	2	16
2500			40			9			18	32		12
2600			36			8			15	26		8
2700			32			7			11	19		7
2800			30			5			8	17		6
2900			28			4			6	14		6
3000			24			3			5	12		
3100			22							11		
3200			19							10		
3300			17							9		
3400			15							8		
3500			14							8		
3600			12							8		
3700			11							8		
3800			10									
3900			9									
4000			8									
4100			7									
4200			6									
4300			5									

APPENDIX 11

STATISTICAL FORMULAE

1. Determination of Significance of Correlation

Critical $Z = r_s \sqrt{(n-1)}$.

Critical $Z > 1.96$ indicates correlation is significantly different from 0 at $p<0.05$ level.

2. 95% Confidence Interval for Sensitivity/Specificity

The 95% Confidence Interval (95% CI) is the range of values within which there is 95% certainty that the true parameter value lies.

95% CI = P+/- 1.96 S.E. P

SE= $\sqrt{(P(1-P)/n)}$

3. Determination of Significant Difference between Sensitivities and Specificities

$T = (p1-p2) / S.E. (p1-p2)$

$S.E. (p1-p2) = \sqrt{(p(1-p) (1/n1+1/n2))}$

$T > +/- 1.96$ indicates significant difference at $p<0.05$ level.

4. The Kruskal-Wallis Test

This test is the non-parametric alternative to the one-way analysis of variance. It is used to compare the distributions of more than two independent groups of observations.

5. The Mann-Whitney *U* Test

This is a non-parametric test used to compare the distributions of two independent groups of observations.

6. The Wilcoxon Signed Ranks Test

This is a non-parametric test comparing paired observations.

7. Standard Error of the Mean (SEM)

A measure of the precision of the sample mean. It is the standard deviation of the sampling distribution of the mean.

8. The Shapiro-Wilk Statistic

This test determines whether or not data are Normally distributed.